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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 47/48, G01N 33/543, C07H 13/04 | A2

(11) International Publication Number: WO 98/46270

(43) International Publication Date: 22 October 1998 (22.10.98)

(21) International Application Number: PCT/US98/07171

(22) International Filing Date: 9 April 1998 (09.04.98)

(30) Priority Data:

60/043,781	11 April 1997 (11.04.97)	US
60/043,826	14 April 1997 (14.04.97)	US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

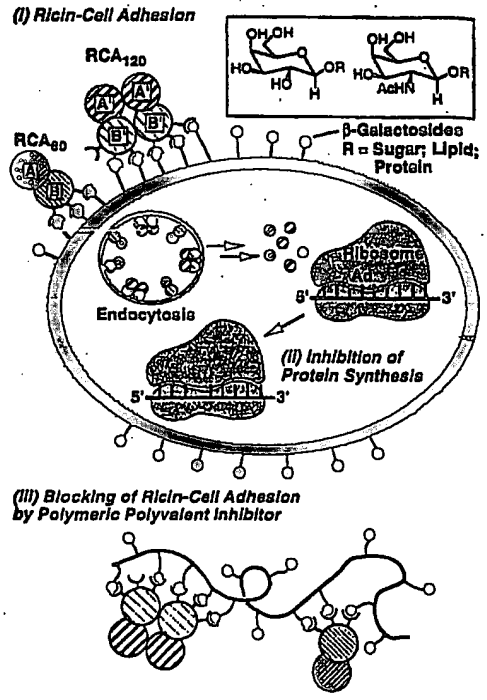
Published

Without international search report and to be republished upon receipt of that report.

(54) Title: MOLECULES PRESENTING A MULTITUDE OF ACTIVE MOIETIES

(57) Abstract

Pharmaceutical compositions for polyvalently presenting an agent for therapy are described. The pharmaceutical compositions contain a polyvalent presenter. In one embodiment, the polyvalent presenter has a formula as follows: $(Y)-(X-A)_n$, wherein Y is a framework, X is a direct bond or a linker, A is a presented functional group, and n is greater than ten and is an integer selected such that the presented groups can interact with a plurality of target binding sites. The composition also can include a pharmaceutically acceptable carrier. Alternatively, the presenter itself can serve as its own pharmaceutically acceptable carrier. Methods for treating diseases or conditions also are described. The methods involve administering to a subject a plurality of groups A such that the treatment occurs. The treatment occurs by the interaction of a polyvalent presenter with a plurality of target binding sites B. Other aspects of the invention include polyvalent presenters packaged with instructions for use in the aforementioned methods and methods for designing polyvalent presenters which are useful within the methods of this invention. The polyvalent presenters disclosed herein provide for specificity in binding, which has a number of advantages. Furthermore, the polyvalent presenters permit positive and negative interactions.



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MOLECULES PRESENTING A MULTITUDE OF ACTIVE MOIETIES

Government Funding

This work was supported, in part, by the NIH Grants GM30367 and GM39589. The NMR facilities at Harvard were supported by NIH grant 1-S10-RR04870-01 and NSF grant CHE88-14019. The government may have certain rights to this invention.

Related Applications

This application claims priority to U.S. Provisional Applications Serial Nos. 60/043,781 filed April 11, 1997, and 60/043,826 filed April 14, 1997, which are incorporated herein by reference. This application is related to an application entitled "Polyvalent Presenter Combinatorial Libraries and Their Uses," U.S. Provisional Application Serial No. 60/043,288 filed on April 11, 1997, and an application entitled "Polyvalent Presenter Combinatorial Libraries and Their Uses," U.S. Provisional Application Serial No. 60/043,918 filed on April 15, 1997.

Background of the Invention

Polyvalency is pervasive in biology and is important to the functioning of biological systems. Polyvalency is the interaction, e.g. occurring simultaneously, between two discrete species through multiple pairs of individual ligand-receptor binding events. (See Figure 1). Interactions of ligands with receptors occur throughout biology. Ligands include molecules that convey information in biological systems or that are acted on by proteins. Examples of types of ligands are drugs, hormones, signaling molecules, cell surface markers, toxins, enzyme substrates, bioregulators, neurotransmittors and lymphokines. Receptors include molecules that interact and receive the information from the ligands. Most receptors are proteins and include protein receptors, antibodies, and enzymes. Some receptors are nucleic acids and include the regulatory regions of DNA and RNA.

Many drugs are ligands that interact with a single receptor (some drugs are receptors that interact with a single ligand). In biology, many important events derive from the simultaneous interaction of multiple ligands with multiple receptors. Polyvalent interactions pervade biology. Polyvalency typically is involved in interactions that occur at cell surfaces

and/or many interactions involving groups of receptors or clusters of receptors. Polyvalency also can be important in macromolecular interactions involving multiple points of attachment simultaneously.

The concept of polyvalency has been explored. It has been explored in the context of studies intended to provide insight regarding the mechanism for adhesion of the influenza virus to the surface of cells. (Whitesides, *et al.*, *J. Med. Chem.* 1995, 38, 4179-4190; Sigal, *et al.*, *Journal of the American Chemical Society* 1996, 118, 16, 3789-3800). Kiessling, *et al.*, (*Chemistry and Biology* 1996, Vol. 3, No. 2, 71-77) created structural templates for the generation of multivalent carbohydrate displays to study and modulate biological recognition events. Kiessling *et al.* viewed these as "chemical tools available to explore multivalent protein-saccharide interactions". In a section of the reference entitled "Where do we go from here?" Kiessling *et al.* sets forth that "[a]s well as being useful to explore biological recognition processes, multivalent carbohydrates can be used as probes for biological function". Matrosovich (*FEBS LETTERS* 1989,252,1, 1-4) proposed the use of polyvalent inhibitors for inhibiting microbial attachment. Matrosovich sets forth that "[t]o produce such polyvalent structures, one might utilize some principles of the design of the well known 'drug delivery systems', for example, coupling the monovalent inhibitory active molecules in multiple copies to soluble biocompatible polymers or microparticulate carriers". Matrosovich further sets forth that" [t]he correctness of and the prospects for the practical use of this approach to the design of antimicrobial agents could be evaluated in the future...".

Summary of the Invention

To date, "high affinity, specific binding events have dominated most thinking about receptor-ligand interactions" (see page 71 of Kiessling *cited supra*). Typically, attempts to optimize receptor-ligand interactions have focused on the binding capability or the specificity of a particular ligand with a particular receptor-binding site in an individual binding event. For example, a ligand was selected for interaction based upon its known favorable binding capability or a ligand believed to be a weak binder was avoided or chemically modified to enhance its binding capability before being selected for use. Weakly binding ligands do not have to be avoided as components of the polyvalent presenters of the present invention.

The present invention is based, at least in part, on our viewing of receptor-ligand interactions and polyvalency in a non-conventional, global manner based on an understanding

of how a multicomponent polyvalent presenter interacts with a collection of target binding sites. Our approach differs from the conventional manner of viewing such interactions on a more individualistic basis as separate receptor-ligand interactions. This non-conventional, global manner of viewing receptor-ligand interactions and polyvalency in biological systems led us to the realization that polyvalency can be used as a basis, e.g., a primary basis, for rational drug design and even further has universal applications for treating many different diseases or conditions. This non-conventional, global manner of viewing receptor-ligand interactions has even further led us to the realization that the choice of a particular ligand based on its individual binding capability need not be the most important parameter in designing a polyvalent drug.

The polyvalent presenters of the invention are formed by constructing and arranging a plurality of groups A (e.g., ligands), which may be the same or different, on a framework (e.g., a polymeric backbone) forming a polyvalent presenter for treating a disease or condition. The construction and arrangement of the groups A on the framework can be made based on a therapeutic effect due to the blanketing of a collection of target binding sites B or an array of target binding sites B (e.g., clustered receptor binding sites on a cellular surface) or multiple type of binding sites for a single ligand and multiple types of binding sites for multiple types of ligands with the attached polyvalent presenter(s) within a subject. The blanketing of a collection of target binding sites B occurs as the result of the conformal interface interaction of a polyvalent presenter with a collection of target binding sites B. The blanketing of an array of target binding sites occurs as the result of the conformal interface interactions with a plurality of collections of target binding sites B. The blanketing is not intended to be limited to a continuous barrier and is discussed in further detail in the Detailed Description below.

For the purpose of illustration, the blanketing effect will be discussed in detail as it pertains to a polyvalent presenter having a polymeric framework. As shown in Figure 2, a polyvalent presenter **P** having a polymeric framework **1** is capable of assuming a configuration which conforms to an interface **3**, e.g., surface, containing a collection of target binding sites **5** as it interacts. One or more attached conformed polyvalent presenters essentially blanket an array of target binding sites B (made up of more than one collection of target binding sites B) enhancing or providing the therapeutic effect of the polyvalent presenter(s). The blanket can be a physical blanket, e.g., a physical covering up of the target

binding sites B with the polyvalent presenter and/or a steric blanket, e.g., a crowding of the target binding sites B with multiple groups A of the polyvalent presenter. The blanket does not have to be a continuous or a tightly conforming barrier but can be a non-continuous or loosely conforming barrier as shown in Figure 2, and the blanket does not have to cover the target binding sites completely. In some instances, the blanket will be a gel layer that conforms to the surface containing the array of target binding sites B.

The present invention even further pertains to methods for treating a disease or condition. In one embodiment, the method involves administering to a subject a plurality of groups A such that the treatment of the disease or condition occurs by the blanketing of a collection of or an array of target binding sites B within the subject. The blanketing of a collection of target binding sites B within the subject occurs via the conformal interface interaction of a single polyvalent presenter with a collection of target binding sites B. The blanketing of an array of target binding sites B occurs via the conformal interface interactions of a plurality of polyvalent presenters with collections of target binding sites B. In other embodiments, the methods involve the use of polyvalent presenters that meet certain criteria or the use of polyvalent presenters having particularly selected components, e.g., groups A, frameworks or linkers.

The present invention also pertains to pharmaceutical compositions for polyvalently presenting an agent for therapy. The pharmaceutical compositions contain a polyvalent presenter. The polyvalent presenter can have a formula as follows:

$$(Y)\text{-}(X\text{-}A)_n$$

wherein Y is a framework, X is a direct bond or a linker, A is a presented functional group and each A may be the same or different, and n is greater than ten and is an integer. The presenter is selected such that the presented groups A can interact with a collection of target binding sites B. The composition also includes a pharmaceutically acceptable carrier. The presenter itself can serve as its own pharmaceutically acceptable carrier. In one embodiment, the polyvalent presenter is made, e.g., n is selected and the -(X-A) moieties are attached to Y, such that the polyvalent presenter conforms to an interface containing a collection of target binding sites B and blankets the collection of target binding sites B upon administration to a subject. In another embodiment, X is a linker group that is an independent moiety and is not part of Y or A and n is greater than ten and is an integer.

Such a presenter is selected such that the polyvalent presenter conforms to a collection of target binding sites B upon administration to a subject. In yet another embodiment, Y is a polymeric framework, A is a presented functional group and X is a linker group and n is greater than ten and is an integer. This structure is selected such that the polyvalent presenter conforms to a collection of target binding sites B upon administration to a subject.

Another aspect of the present invention is a method of modulating adhesion between a plurality of a surface-bound biological molecule and a plurality of a second molecule. The method comprises providing in combination a plurality of a surface-bound biological molecule and a polyvalent presenter. The polyvalent presenter comprises a framework to which is attached a plurality of a third molecule. The third molecule modulates the adhesion between the surface-bound biological molecule and the second molecule by simultaneous entropically enhanced binding of the third molecule to a portion of the plurality of the surface-bound biological molecule and by sterically blocking by the framework of a portion of the plurality of the surface bound biological molecule to which the third molecule is not bound.

Another aspect of the present invention is a method as described above wherein the polyvalent presenter is a non-saccharide and has an enhancement factor β greater than about 10.

Another aspect of the present invention is a polyvalent presenter that is pAA(Gal-β), pAA(Gal-α), pBMA(Gal-β) or pBMA(Gal-α) as defined below.

Another aspect of the present invention is a method for preventing the adhesion of ricins to erythrocytes. The method comprises contacting the ricins with an effective amount of a polyvalent presenter selected from the group consisting of pAA(Gal-β), pAA(Gal-α), pBMA(Gal-β) and pBMA(Gal-α).

Another aspect of the present invention is a method for preparing a polyvalent presenter selected from the group consisting of pAA(Gal-β) and pBMA(Gal-β). The method comprises reacting Gal-β_O-L_1NH_2 with poly(*N*-acryloyloxysuccinimide) or poly(butadiene-co-maleic anhydride and quenching the reaction.

Another aspect of the present invention is a method for preparing a polyvalent presenter selected from the group consisting of pAA(Gal-α) and pBMA(Gal-α). The

method comprises reacting Gal-α_C-L_2NH_2 with poly(*N*-acryloyloxysuccinimide) or poly(butadiene-co-maleic anhydride) and quenching the reaction.

Another aspect of the present invention is a polyvalent presenter that is pAA(GlcNAc-β).

Another aspect of the present invention is a method for inhibiting fertilization in a subject comprising administering to the subject an effective amount of a polyvalent presenter that is pAA(GlcNAc-β).

Another aspect of the present invention is a method for preparing a polyvalent presenter that is pAA(GlcNAc-β). The method comprises reacting GlcNAc-β-L_1NH_2 with poly(*N*-acryloyloxysuccinimide) and quenching the reaction.

Brief Description of the Figures

Figure 1 is a schematic depicting both a monovalent and polyvalent reaction.

Figure 2 is a schematic depicting the "blanketing" of a polymeric polyvalent presenter(s) over a collection of binding sites and an array of target binding sites B.

Figure 3 is a schematic depicting ricin-cell interactions.

Figure 4 is a schematic depicting a strategy for induction of acrosome reaction of sperm.

Figure 5 is a schematic depicting the synthesis of polymeric polyvalent galactosides.

Figure 6a is a schematic depicting plots of agglutination inhibition activity of polymeric polyvalent galactoside versus mole fraction of Gal of the polymer against RCA_{120}.

Figure 6b is a schematic depicting plots of agglutination inhibition activity of polymeric polyvalent galactoside versus mole fraction of Gal of the polymer against RCA_{60}.

Figure 7 is a schematic depicting the structure of polyvalent polymeric N-acetylglucosamine and galactoside.

Figure 8 is a schematic depicting the induction of acrosome reactions of mouse sperm by monovalent and polyvalent GlcNAc.

Figure 9a is a schematic depicting the induction of acrosome reactions of mouse sperm by polymeric polyvalent GlcNAc.

Figure 9b is a schematic depicting a plot of acrosome-reacted sperm (%) obtained with pAA(GlcNAc).

Figure 10 is a schematic depicting a plot of the inhibition of sperm-egg binding by polymeric polyvalent GlcNAc.

Figure 11a is a schematic depicting the generation of pMVMA(NeuAc).

Figure 11b is a schematic depicting the generation of pMVMA(NeuAc;R).

Figure 11c is a schematic depicting the generation of pAA(Gal).

Figure 11d is a schematic depicting the generation of pBMA(Gal).

Figure 12a is a schematic depicting the generation of pAa(SLe^x).

Figure 12b is a schematic depicting the generation of pAA(Bacitracin;R).

Detailed Description of the Invention

DRUG DESIGN

The present invention pertains to a method for designing a polyvalent presenter for administration to a subject for treating a disease or condition. The method involves constructing and arranging a plurality greater than ten of groups A on a framework forming a polyvalent presenter for treating a disease or condition. The construction and arrangement is made based on the designed presenter's ability to provide a therapeutic effect due to the blanketing of a collection of target binding sites B. In a preferred embodiment, an array of target binding sites B within the subject is blanketed with one or more of the polyvalent presenters. The blanketing results from the conformal interface interaction of a polyvalent presenter with a collection of target binding sites B or the conformal interface interactions of a plurality of polyvalent presenters with collections of target binding sites B.

The "polyvalent presenter" of the present invention is described in detail below and includes multi-component molecules having eleven or more presented groups A (capable of binding to eleven or more target binding sites B) attached to a framework. Briefly, a polyvalent presenter can have a formula as follows:

$$Y\text{-}(A)_n$$

wherein "Y" represents a framework; "A" represents a presented, functional group; and "n" represents an integer greater than ten selected such that the presented group can interact with the plurality of binding sites B.

The language "disease or condition" includes diseases or conditions whose treatment with the polyvalent presenters of the present invention occurs via a polyvalent interaction, e.g., the interaction of groups A with a polyvalent receptor comprising target binding sites B. Diseases or conditions which are intended to be part of this invention typically can be characterized by an interaction between binding sites B and groups C, wherein both B and C are polyvalent. As used herein, "B" represents a plurality of binding sites and "C" represents a moiety or group associated with the disease or condition in nature and which interacts with B. In some embodiments, groups C can be the same as groups A, which are presented on the polyvalent presenter of the invention, leading to competition between poly (C) and poly (A) for binding sites B. In other embodiments, groups C can be different from groups A. While binding sites B are naturally occurring, groups C can be either synthetic or natural. For example, C can be a naturally occurring sugar, peptide, protein (e.g., a ligand for a cell surface receptor, such as a viral antigen) or can be synthetic (e.g., can be a group presented on a silicon implant or a prosthesis). Groups C can be on a surface, or can be in solution, but must be polyvalently presented. The language "disease or condition" also is intended to include conditions or diseases identified as involving, or being affected by, a naturally occurring polyvalent molecule, e.g., the α_2 macroglobulin molecule that inhibits interaction between the influenza virus and a target cell. While biological processes are complex, one of ordinary skill in the art would recognize whether or not a disease or condition involves a polyvalent interaction by examining whether or not the specific point at which the polyvalent presenter acts (i.e., the specific event targeted for intervention with a polyvalent presenter) was associated with an interaction between poly(C) and poly(B). Examples of diseases and conditions are described in detail below under the heading "**METHODS FOR TREATING DISEASES OR CONDITIONS**".

The term "subject" includes mammals susceptible to or having the disease or condition being treated or targeted for treatment. As such the invention is useful for the treatment of humans, domesticated animals, livestock, zoo animals, etc. Examples of subjects include humans, cows, cats, dogs, goats, and mice. In preferred embodiments the present invention is used to design drugs for treating human subjects.

The language "treating a disease or condition" includes the treatment of a subject having the disease or condition amenable to treatment with a polyvalent presenter of

the present invention or prophylaxis of such a disease or condition. The term "treatment" includes providing a beneficial effect to the subject, e.g., a significant reduction of at least one symptom or manifestation of the disease or condition. Therefore, "treatment" is meant to include anything along a continuum from amelioration of at least one of the symptoms of the disease or condition to cure of the disease or condition. The treatment also is intended to include the use of a polyvalent presenter, alone, in conjunction with other polyvalent presenter(s) or even in conjunction with other treatments with agents which are not polyvalent presenters.

The language "constructing and arranging a plurality of groups A on a framework" includes the manipulation of various components of the polyvalent presenter to produce a presenter capable of performing its intended function in the treatment of a disease or condition. The manipulations can be made based upon a viewing of the interaction(s) between the groups A and the collection of target sites B in a global manner. The manipulation includes the positioning, sizing and selection of various components, e.g. framework, linkers, groups A, of the polyvalent presenter. For example, such language is intended to cover the positioning of the groups A and the framework relative to each other or relative to an optional linker molecule used to attach the groups A to the framework or a backbone linker used to connect monomers of the framework. The language also is intended to include the selection of particular types of groups A, framework, and/or linker.

A framework can be selected for its ability to form a particular type of "blanket" over a collection of target binding sites B when it is part of a polyvalent presenter. For example, some frameworks will form gel-like physical barriers over a collection of binding sites B. A framework also can be selected based upon a different characteristic that would be desirable to have in the polyvalent presenter being designed. A framework can be selected based on its "flexibility" and/or its ability to impart flexibility to the polyvalent presenter even after attachment of groups A.

The groups A also can be selected based on their ability to impart desirable characteristics into the polyvalent presenter. It is important to emphasize that the binding capability of the group A is a factor in it's selection process but at least part of the present invention is the recognition that a weakly binding group A can be useful in its polyvalent form.

The positioning of the groups A on the framework also is intended to be part of the "constructing and arranging" of the polyvalent presenter. The positioning can be made based on the known or predicted, e.g. using molecular modeling of the polyvalent environment, spatial arrangement of a collection of target sites B. The positioning can be along several different directional axes relative to the framework. For example, the target sites B may be spaced an average of 10 angstroms apart from their neighboring target binding sites B and therefore the groups A can be spaced or positioned along the framework in the horizontal direction appropriate for providing access to neighboring target binding sites B. It should be understood that the groups A do not have to be positioned to match the distance between neighboring target binding sites but rather are spaced appropriately to provide access. Other factors besides distance are considered when positioning the groups A on the framework such as the flexibility of the linker and the contour of the interface containing the target binding sites. For example, a flexible linker can make adjustments *in vivo* for providing access to the target binding sites even if the distance between the groups A does not exactly match the distance between the neighboring target binding sites B or if the contour of the interface is curved rather than flat. The depth of the binding pocket, e.g. 2 to 20 angstroms, also may be known or predictable allowing for the positioning of the groups A along an axis substantially perpendicular to the framework, e.g., a particular length of linker can be used.

The "constructing and arranging" also is intended to include the selection of a type and length of linker that attaches the groups A to the framework. The length of the linker can be selected based on its ability to present the group A to a target binding site B within a binding pocket having a known or predicted depth and/or diameter. The chemical nature of the linker, e.g. hydrophobicity or hydrophilicity, also can be selected based on knowledge regarding the environment surrounding the target binding site, e.g. the linker may have to pass through a channel or environment known to be hydrophobic or hydrophilic. The linker also may be selected based on its ability to impart a desired property into the polyvalent presenter, e.g. flexibility.

The language "therapeutic effect" is intended to include the ability of the polyvalent presenter to perform its intended function, e.g. treat or prevent the disease or condition. The therapeutic effect of the polyvalent presenter can be measured using an art-recognized assay for the particular disease or condition targeted for treatment by the

polyvalent presenter being designed or by using an assay designed using art-recognized techniques and/or by an assay described herein which would not fit into one of these categories. The therapeutic effect is considered "due to the blanketing" when the effect is derived from the ability of the polyvalent presenter to conform to and engage in multivalent binding with the collection of target binding sites B.

The term "blanketing" includes both physical blanketing or the physical covering up of the target binding sites B and steric blanketing, e.g., the steric smothering of target site(s) B and/or by enhancing the occupancy of site B by ligand A. Examples of physical blanketing include the formation of a gel-like layer or barrier over the collection of binding sites B which prevents access to such sites by groups other than those present on the polyvalent presenter. The physical barrier also can be the spaghetti-like barrier depicted in Figure 2. In either case the formation of a gel-like layer or barrier or a spaghetti-like barrier results biospecifically by the specific binding of at least some of the groups on the polyvalent presenter with a corresponding binding partner on the target.

The "steric blanketing" of a target binding site B is the surrounding of the binding site B with groups A attached to a framework in a velcro-like manner. A group A can be bound to the binding site but other group A's can be sterically blocking the same binding site B or a neighboring target binding site.

The language "collection of target binding sites B" includes the binding sites B over which a single polyvalent presenter molecule conforms. For example, if a polyvalent presenter has ten groups A attached to a framework then the collection of binding sites would be that span of binding sites B over which this molecule conforms, e.g., ten to twenty binding sites although only as many as two binding sites may actually be occupied. It should be noted that not all binding sites B have to interact with a group A and some sites may be left unoccupied.

The language "an array of target binding sites B" includes more than one collection of target binding sites B on an interface. The binding sites B within the array do not have to be equally spaced or positioned but rather can be randomly positioned in various directions along different directional axes relative to the interface, e.g., depending on such factors as the contour of the interface and the clustering configuration of the binding sites. For example, the contour of the interface can be flat or can be curved and also can be mobile.

The language "conformal interface interaction" includes an interaction which occurs while a polyvalent presenter is in a conformed configuration over a collection of target binding sites B at an interface, e.g., the molecular region accessible to groups A. The interface can be, but is not limited to a surface, but is intended to include the boundary of interaction. The term "conformal" is intended to include situations wherein the interface between the polyvalent presenter and the surface is close or tight with respect to all or a part of the surface. In the latter instance the polyvalent presenter may be close to the surface in some parts and less close in other parts of the surface. The term "interaction" is intended to include both positive and negative interactions as discussed in detail below. The polyvalent presenters of the present invention conform to an interface, e.g., a surface such as a cell surface, by being flexible enough to adjust to the contour of the interface. This conformation can be along a span of a particular number of target binding sites B. Preferably, this span is at least about 50, at least about 100, at least about 1000, up to about at least about 10^6 target binding sites B. Ranges intermediate to the above recited values, e.g., at least about 50 to about 1000, or at least about 1000 to about 10^6, also are intended to be part of this invention. For example, ranges of span values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included. The above ranges are intended to include both sites within a collection of target binding sites B and sites within an array of target binding sites B.

The term "interaction" is intended to include both negative interactions, e.g., inhibitory, e.g., influenza, and positive interactions, e.g., interactions that result in the transduction of a signal or an interaction that brings two surfaces together. For example, the polyvalent presenter can bind to a collection of target binding sites B, e.g., on a cell surface, and transduce a signal within a cell or induce the cell to expend energy. Examples of effects of such an interaction include apotosis (programmed cell death), clonal expansion, e.g., B-cells or T-cells multiplication, cell migration, e.g., movement of a cell in some direction, release of soluble molecules such as hormones, e.g., insulin, cytokines, e.g., Il2; prostaglandins, endocytosis or pinocytosis or exocytosis, active transport (inward or outward) of some substance, induction of electrical activity, or differentiation or dedifferentiation of a particular cell. Modulation or treatment of all such effects with a

polyvalent presenter is intended to be part of this invention. In some embodiments, the interaction is not anti-adhesive.

The term "steric stabilization" refers to a mechanism by which a polyvalent presenter sterically inhibits the close approach of two surfaces by binding biospecifically to one of the surfaces. This is distinguished from stabilization resulting from the addition of water-swollen polymers to colloidal mixtures, which is utilized to keep colloidal particles in solution. In such cases, the molecule binds non-specifically to the surface to be stabilized. See, for example, A. Sung and I Piirma "Electrosteric stabilization of polymer colloids" Langmuir 1994, 10, 1393-8; U Genz, B D'Aguanno, J Mewis and R Klein "Structure of sterically stabilized colloids" Langmuir, 1994, 10, 2206-12. The present concept is also distinguished from grafting polymers to the surface of colloids or liposomes. In the latter case, steric stabilization is attained by covalent attachment of a molecule to the surface to be stabilized. Polyetheylene glycol is a prototypical example. See, for example, K Zhulina, O Borisov and V Priamitsyn "Theory of steric stabilization of colloid dispersions by grafted polymers" Colloid and Interfacial Science 137:495-511, 1990.

The language "polyvalent presenter" includes multi-component molecules having eleven or more presented groups A, which are capable of binding to corresponding target binding sites B, and which groups A are attached to a framework. A polyvalent presenter of the present invention can be exemplified by the following structure:

$$(Y)\text{-}A_n$$

In certain embodiments, a polyvalent presenter of the present invention can be represented by the following structure:

$$(Y)\text{-}(X\text{-}A)_n$$

In the above representations "Y" represents a framework; "A" represents a presented, functional group; "X" represents an optional linker group which can be used to attach groups A to the framework Y and "n" represents an integer greater than ten selected such that the presented group can interact with the plurality of binding sites B.

The integer "n" is selected such that a sufficient number of groups A are presented for treatment of a disease or condition. The integer "n" is further selected to allow the polyvalent presentation of a plurality of groups A. The integer "n" is greater than ten, preferably greater than ten to about 10^6, more preferably about 50 to about 10^6, about 100 to about 10^6, or about 1000 to about 10^6. The ranges of "n" values intermediate to those listed also are intended to be part of this invention, e.g., greater than ten to about 100, greater than ten to about 1000, about 100 to 1000, and about 1000 to 100,000. For example, ranges of "n" values using a combination of any of the above values recited as upper and/or lower limits are intended to be included.

The language "polyvalent presentation" or "polyvalent manner" is art-recognized and includes the polyvalent display of A, B, or C, such that the poly A, B, or C functions differently than its monovalent equivalent. For example, polyA may show a biological effect that is either positively cooperative or negatively-cooperative or non-cooperative from monoA. In addition, the polyvalent presenters may act independently of cooperativity as demonstrated by the β factor as explained more fully below. Polyvalent presentation or polyvalent manner includes the use of polyvalent presenters which differ from the slow release compounds or drug delivery systems known in the art. Polyvalent presenters are designed to function polyvalently, not to release monovalent groups that mediate the drugs pharmacological activity. In addition, because of the release and diffusion of monovalent groups, slow release compounds typically act at a site that is different from the site of administration and this does not have to be true in the case of polyvalent presenters.

FRAMEWORKS

The term "framework" (Y) includes a support structure or backbone made of a material having a molecular weight greater than about 10,000 to which, a plurality of groups A can be attached and can be presented polyvalently. The attachment can be by a means that allows the polyvalent display of the group on the framework. In certain embodiments, groups A are attached to a framework prior to administration to a subject. In other embodiments, groups A are administered to a subject and assembled on a framework *in vivo* to form a polyvalent presenter of the invention, e.g., by self assembly. The framework component must be of sufficient mean hydrodynamic radius to span the distance between adjacent receptors, and will be 100 angstroms or greater. These dimensions permit the plurality of functional groups A attached to the framework to simultaneously bind to the target receptors, for example cell surface receptors. One of ordinary skill will recognize that the mean hydrodynamic radius of a polymer may be estimated crudely using standard statistical mechanical methods.

In some embodiments, the "backbone" of a framework can further comprise linkers (hereinafter backbone linkers) for joining monomer units of the framework together. Specific backbone linkers will be discussed in detail below in the context of polymeric frameworks. In certain embodiments, the backbone linkers are cleavable, e.g. the backbone linkers are hydrolytically labile.

The types of frameworks useful within the polyvalent presenter are those capable of having groups A attached thereto and capable of polyvalently presenting the groups A. The frameworks used for an *in vivo* purpose also are suitable for *in vivo* administration or are available *in vivo* for assembly. Examples of types of frameworks include, but are not limited to, polymers, liposomes, micelles, colloids, dendrimers, and biological particles. These types are discussed briefly below and in more detail even further below under the description of covalent and noncovalent frameworks. The detailed description of each of these frameworks are provided under the headings of covalent and noncovalent frameworks only for ease of discussion and should not be construed as limiting the scope of frameworks. It should be understood that the present invention is intended to encompass all types of frameworks capable of polyvalently presenting groups A as described herein.

The terms "polymer" or "polymeric" are art-recognized and include a structural framework comprised of repeating monomer units. To qualify, the polymeric framework must further be capable of polyvalently presenting "A" groups such that the treatment of a disease or condition occurs. The terms also include copolymers and homopolymers e.g., synthetic or naturally occurring. Linear polymers, branched polymers, and cross-linked polymers are also meant to be included. In a preferred embodiment, the polymer is not dextran.

The terms "liposome", "micelles", and "colloids" are art-recognized. These terms also include derivatized versions, e.g., liposome derivatives, cross-linked liposomes and the like.

The term "dendrimer" is art-recognized. Dendrimers include a specific subclass of branched polymers that possess multiple generations. In dendrimers, each generation creates multiple branch points. In a preferred embodiment the framework is not a dendrimer.

In certain embodiments, frameworks of the present invention can comprise "biological particles". The term "biological particle", includes both covalent molecules, e.g., sugars, proteins, lipids, small molecules, protein aggregates, and nucleic acids, and noncovalent particles, e.g., modified cells (e.g., which have been derivatized, modified chemically or transfected with an exogenous nucleic acid) or modified viruses, e.g. viral particles. The use of "biological particles" as frameworks is distinguished from such particles as they occur in their natural state because the subject frameworks are modified to polyvalently present functional groups A.

Covalent Frameworks

In one embodiment, the monomeric units of a framework can be joined covalently. Exemplary covalent frameworks include crosslinked liposomes, biological particles (e.g., sugars, proteins, peptides, lipids, or small molecules), and polymers. (see e.g., Siraganian, R. P., *et al., Immunochem.* 1975,*12,* 149-155; Wofsy, C., *et al., J. Immunol.* 1978,*121,* 593-601; Barlocco, D., *et al., Farmaco* 1993.*48.* 387-96; Castagnino, H. E., *et al., Jpn. Heart J* 1990, *31,* 845-55; Costa, T., *et al., Biochem. Pharmacol* 1985, *34,* 25-30; Dembo, M., *et al., J. Immunol* 1979, *122,* 518-28; Holliger, P., *et al., Proc. Natl. Acad. Sci. U. S. A* 1993, *90,* 6444-8; Piergentili, A., *et al., Farmaco* 1994, *49,* 83-7; Portoghese, P. S.;

et al., J. Med Chem 1991, *34,* 1292-6; Kizuka, H. and Hanson, R, N. *J. Am. Chem Soc* 1987. *30,* 722-6).

In certain embodiments proteins, e.g., albumin can be used as a system for presenting large numbers of groups; (Roy, R.; Lafemere, C. *A. Can. J. Chem.* 1990, *68,* 2045-2054) thus mimicking natural glycoproteins.

In other embodiments, polymers can be used as the framework for a polyvalent presenter. Polymers are a versatile framework system. (Spaltenstein *et al.* 1991, *J. Am. Chem. Soc.* 113:686; Mammen *et al.* 1995. *J. Med. Chem.* 38:4179). In a preferred embodiment, the groups A of the present invention are attached to a framework comprising a polymeric backbone by a linker group. In certain embodiments reactive polymers can be used in a "framework" of the present invention as described in more detail below.

Polymers can be prepared using methods known in the art (Sandler, S. R.; Karo, W. *Polymer Syntheses;* Harcourt Brace: Boston, 1994; Shalaby, W.; Ikada, Y.; Langer, R.; Williams, J. *Polymers of Biological and Biomedical Significance (ACS Symposium Series 540;* American Chemical Society: Washington, DC, 1994). Polymers can be designed to be flexible; the distance between the bioactive side chains and the length of a linker between the polymer backbone and the group can be designed and controlled.

Polymers provide a number of advantages as a framework. They can be designed to allow multiple groups A to bind simultaneously to multiple group B binding sites with minimal unfavorable strain. Polymeric, polyvalent presenters are easily, rapidly and convergently synthesized (Spaltenstein *et al.* 1991, *J. Am. Chem. Soc.* 113:686; Mammen *et al.* 1995. *J. Med. Chem.* 38:4179). Polymers also allow modulation of various physical properties of the presenter, for example, conformal flexibility; solubility; hydrophilicity, and the modulation of conformation and flexibility in solution through variations in temperature and ionic strength.

The chemistry of high molecular weight polymers is a well-developed science, and organic polymers provide a very important class of compounds to use for polyvalent presentation. These compounds have high molecular weights, and can present very large numbers of copies of the group; they can present more than one group simultaneously; their transport across biological membranes is typically limited, and, thus, their lifetime in particular compartments can be controlled *in vivo.* Polymeric frameworks offer a variety of easily synthesized macromolecules, and access to a range of biological activities.

In preferred embodiments, modified polymeric materials for use in the present invention have low antigenicity and low toxicity. In preferred embodiments polymeric frameworks can be selected to be compatible with water, will be capable of having varied molecular weight, and will be capable of having a range of different groups attached to the polymer backbone. Polymer backbones of the present invention may also be selected for ease of synthesis.

Intrinsically biocompatible polymers containing functional groups appropriate for the addition of side chains are preferred (Shalaby, W.; Ikada, Y.; Langer, R.; Williams, J. *Polymers of Biological and Biomedical Significance (ACS Symposium Series 540);* American Chemical Society: Washington, DC, 1994). Exemplary polymers include polyethylene oxide or polyethylene glycol (Harris, J. M. *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications.;* Plenum: New York, 1992; Horton, D. *Advances in Carbohydrate Chemistry and Biochemistry;* Academic Press: San Diego, 1995) as well as, for example, derivatives of acrylamide and N-vinylpyrrolidone, linked oligomers of oligoethylene glycol, linked oligomers of dextran, and others.

Other preferred frameworks for use with the present invention have demonstrated utility, e.g., as plasma extenders, drug excipient or binders, food additives, or as an inert or erodible materials used in vivo. For example, poly(ethylene glycol), poly(lactic acid), poly(glycolic acid), and poly(vinyl pyrrolidone) can be used.

Preferred polymers for use in the synthesis of polyvalent presenters contain reactive groups, such as activated carboxylic acids. A number of synthetic and naturally occurring polymers contain carboxylic acid functionality or are capable of being modified with such, and these have been used *in vivo*. Such polymers are capable of forming covalent linkage with a presented group A, e.g., an amide bond. Polymers containing internally cyclized carboxylic acid functionality, such as anhydride or succinimide groups, are especially desirable. Other preferred polymers include subunits derived from maleic anhydride or malic acid. Exemplary copolymers include styrene-maleic anhydride and alpha-olefin-maleic acid copolymers (such as divinylether-maleic acid). In other embodiments sodium carboxymethylcellulose, chondroitin sulfate and poly (methacrylate/acrylate) materials can be used. In still other embodiments polymers without activated carboxylic acids can be used, e.g., dextran sulfate.

Other exemplary polymeric frameworks include: poly(ester), poly(anhydride), poly(carbohydrate), polyols, poly(acrylate), poly(methacrylate), poly(ether) and poly(amino acid)

Still other exemplary polymeric frameworks include: poly(glutamic acid), poly(aspartic acid), dextran, dextran sulfate, poly(maleic anhydride-co-vinyl ether), poly(succinimide), poly(acrylic anhydride),poly(ethylene glycol), poly(lactic acid), poly(glycolic acid), poly(vinyl pyrrolidone), poly(styrene-maleic anhydride), alpha-maleic acid, hyalauronic acid, sodium carboxymethylcellulose, chondroitin sulfate, poly(acrylate), poly(acrylamide), poly(glycerol), and starch.

It will be appreciated by the skilled artisan that any polymeric material that is capable of presenting a plurality of groups A may be suitable for use in the present invention. Polymers can be modified, e.g., as described above or by derivatization, e.g., with bifunctional cross-linking reagents, to provide functionalities suitable for attaching and presenting A groups, as described in more detail below. In certain situations copolymers may be preferred.

Table 1. Exemplary polymers

Poly(ethylene glycols)	Poly(ethylene-vinyl acetate)
	Poly(acrylamides)
Poly(amides)	Poly(urethanes)
poly(peptides), poly(amino acids)	Poly(methacrylates)
poly(aspartic acid), poly(glutamic acid),	Poly(acrylates)
	Poly(maleic acid copolymers)
poly(lysine), others	Poly(anhydrides)
Proteins(gelatin)	Poly(orthoester)
Poly(esters)	
poly(lactic acid), polylactide	
poly(glycolide)	
poly(caprolactone)	Copolymers
poly(tartrate)	with degradable linkage
	with groups for attachment
Polysaccharides	
cellulose; alginates; starch, dextran derivatives	Encapsulation
	for protection
	for targeting
Poly(N-vinylpyrrolidone)	

Backbone linkers. In certain embodiments it will be desirable to include connecting moieties, "linkers" between monomeric units of the backbone. Exemplary "backbone linkers" can include hydrocarbon, carbamate, amide, ether, thioester, thioether, carbonate, and ester connections. In certain embodiments the backbone moieties can be linked with a cleavable linker. The lifetime of the polyvalent material *in vivo* may in part depend on its molecular dimensions. By placing linkers groups between medium-sized oligomers, and by controlling the *in vivo* stability of these linking groups, the lifetime of the polymers *in vivo* can be controlled. The use of non-functionalized, degradable connectors in the polymer backbone can, therefore, be used to assist clearance of the polymer. The cleavable linker will, in general, be different from that which is used to link the polymer backbone to the group A. Such a cleavable linkage will cause the formation of smaller, polymeric functionalized fragments that will be small enough to clear through the kidney. Degradable linkers can include, e.g., hydrolytically labile linkers including ester, carbonate or oxalate groups.

In certain embodiments, the framework of a subject polyvalent presenter can comprise a dendrimer. Dendrimers are art-recognized and include a class of low molecular weight, highly branched, polymers, e.g. often monodisperse. Dendrimers have the advantage that they are of well-defined molecular structure, and that their solutions are relatively non-viscous for their molecular weight. They can present groups in a densely packed fashion relative to a linear polymer, which can be advantageous for targeting receptors that cluster on cell surfaces. The number of groups A can be precisely controlled in a dendrimer, as well as the regulation of the inter-group distance, conformation and rigidity of the dendrimer molecule. Polysaccharides may belong to a class of dendrimer Sabesan, *et al.*, 1992 *J. Am. Chem. Soc.* 14:8636. In one embodiment the head groups of a dendrimer (Roy, R., *et al.*, *Synthesis and Antigenic Properties of Sialic Acid-Based Dendrimers;* Acs Symp. Ser 1994) can be used as a framework.

Noncovalent Frameworks

A plurality of groups A also can be joined to a non-covalent framework. Exemplary noncovalent frameworks include liposomes, micelles, colloids, protein aggregates, modified cells, and modified viral particles. For example, groups A can be tethered to the head groups of molecules in liposomes, membranes, or surfaces (Kingery-Wood, J. E., *et al.*, *J. Am. Chem. Soc.* 1992,*114,* 7303-7305; Spevak, W., *et al.*, *J. Am. Chem. Soc.* 1993, *115,* 1146-1147; Spevak, W., *et al.*, *J. Med Chem.* 1996, *39*, 1018-1020.).

Liposomes and micelles are art-recognized and include macroscopic particles made up of aggregates of lipids, e.g. surfactants. In one embodiment the polyvalent presenter can present groups on a liposome or micelle (Spevak, W., *et al.*, J. *Am. Chem. Soc.* 1993, *115,* 1146-1147; Charych, D., *et al.*, *Chem. & Biol.* 1996, *3*, 113-120). This system mimics closely the shape of the target cell, and can be designed to present a surface that closely matches that of the target cell in group type and group density. For example, lipid molecules containing groups A, (e.g., neuraminic acid (NeuAc) as polar head groups can be reconstituted into liposomes. Liposomes have favorable biocompatibility; and are fairly easy to synthesize. In addition, liposomes can be designed to act as sensors. Polymerized liposomes can be used to sense the conformational change of the liposome by displaying the change in UV/vis absorption of the internal chromophore (e.g., a cross-linked polydiacetylene) of the membrane. For example, the binding of virus to the liposome could be detected by the shift in color (blue to red) as is art-recognized.

In other embodiments, biological particles including, for example, modified cells or modified viruses can be used as framework for polyvalent presentation of the groups A. Thus, proteins, peptides, polysaccharides, fragments of cell membranes, or modified intact cells (for example, erythrocytes), modified bacterial cells or modified viruses can be used as framework in certain embodiments.

Activated Framework Component

As used herein, the term "activated framework" refers to the framework component as described above, including both covalent and noncovalent framework components, that contain functionality which can be activated, by means of an "activating group," and subsequently reacted with at least one functional group, ancillary group and/or spacer group. Appropriate functionality includes, for example, carboxyl (acid form and salts), hydroxyl, sulfhydryl, amide, carbamate, amino, ketone, aldehyde, olefin, aromatic, *etc.* The polymers may be activated prior to exposing them to the functional group ("preactivation"), or may be activated in the presence of the functional group ("*in situ*").

The activation step can entail derivatizing the polymer with groups capable of undergoing reactions with nucleophiles or electrophiles. Further, it is within the scope of the present invention to activate polymers such that they are able to participate in dipolar additions (*e.g.*, 1,3- and 1,4-dipolar addition), cycloaddition reactions (*e.g.*, Diels-Alder type reactions) and polymerization reactions by cationic, anionic or radical initiated mechanisms.

Carboxyl groups may be activated for reaction with nucleophiles by the use of, for example, cyclic or linear anhydrides, activated esters (*e.g.*, N-hydroxysuccinimide, nitrophenol, 4-hydroxy-3-nitrobenzene sulfonic acid, *etc.*), acid chlorides, imidazolides (*e.g.*, from carbonyldiimidazole), carboxylic acid and esters. Carboxylic acid containing polymers may also be activated by forming adducts between the carboxyl group and agents such as, dicyclohexylcarbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, alkyl chloroformates, chlorosilanes, pyridinium salts and Bu_3N, *etc.*

The selection of appropriate activating groups for the carboxyl functionality will be apparent to those of skill in the art. It will be similarly apparent to those of skill in the art which reaction systems will be amenable to, or will require, *in situ* activation or preactivation.

Hydroxyl groups may be activated by the use of carbonates formed by reaction with, for example, alkyl or acyl haloformates (*e.g.*, *iso*-butylchloroformate, p-nitrophenylchloroformate, *etc.*), cyanogen bromide or phosgene. In aspects utilizing polymers containing vicinal diol groups (*e.g.*, dextran and other polysaccharides) oxidation using periodate compounds can be used to provide reactive carbonyl moieties on the

polymeric backbone. Additional methods of activating polymers bearing hydroxyl groups will be apparent to those of skill in the art.

Polymers bearing sulfhydryl groups can be activated using dithiobispyridyl compounds such as, for example, 2,2'-dithiobis(5-nitropyridine), 2,2'-dithiobis(pyridine), *etc.* Additional methods of use in activating sulfhydryl-bearing polymers will be apparent to those of skill in the art.

It will be appreciated by those of skill in the art that the above activation reactions are advanced as examples only and that many further alternatives to these schemes exist.

Once the polymeric framework is activated, it can be reacted with at least one functional group, ancillary group or spacer group or a mixture thereof. Alternatively, the activated polymer can be reacted with a combination of functional groups, ancillary group and/or spacer groups.

The Functional Group Component

The functional group component of the polyvalent presenter prepared in accordance with the methods of the present invention includes those groups capable of being attached to a framework component, an ancillary group and/or a spacer group, and of being polyvalently presented in a functional manner, *e.g.*, for treating a disease or condition. The functional group components can be the same or different. The language "a plurality of functional group components" or, alternatively, "a plurality of group R^3" is intended to cover more than one functional group component, wherein each functional group within the plurality is independently the same or different. The functional group can be the same or different within categories of types of functional groups, *e.g.*, one functional group can be a carbohydrate and another functional group can be an antibiotic. The functional groups can also be different within the same type or category of functional groups, *e.g.*, two different carbohydrates can be functional groups. In the case of a "homomeric presenter," the functional groups presented are the same, whereas in the case of a "heteromeric presenter," the functional groups presented are different, *e.g.*, from different categories or different within the same category. For ease of description below and in the claims, the nomenclature R^3_1 to R^3_n will be used to denote various members of a plurality of R^3.

As used herein, the functional groups presented by the subject polyvalent presenters are functional. The functional groups also provide their function when attached to the framework components of the polyvalent presenters. As discussed above and reiterated here, this differs from the art-recognized drug delivery systems, *e.g.*, polymeric drug delivery systems, which release their therapeutic agents and wherein the therapeutic agents provide a function in released form. For example, in preferred embodiments of the present invention, the functional groups provide function by biospecifically interacting with eleven or more (*i.e.*, a plurality of) binding sites B to, for example, directly provide a therapeutic effect. In other embodiments, when the polyvalent presenter is, for example, a heteromeric presenter, certain of the functional groups (*e.g.*, R^3_n) may not interact with B, but instead provide another function. For instance, the functional groups may influence the interaction of R^3_1 to binding site B and, thus, function as an enhancer group. In other embodiments, the functional groups (*e.g.*, R^3_n) can be a functional auxiliary group (*e.g.*, an ancillary group) that is capable of influencing the physical characteristics of the polyvalent presenter, *e.g.*, the solubility or ability to cross cell membranes. In other embodiments, the functional groups can provide function by allowing for tracking of the subject presenters, for example, by providing a label that can be detected (*e.g.*, a fluorescent or radioactive tag).

The functional group components of the present invention can be either synthetic or natural. In addition, the functional group components can be categorized based on physical characteristics, *e.g.*, molecular size, *i.e.*, the functional group can be of low, medium or high molecular weight. Examples of natural functional group components include, but are not limited to, naturally occurring sugars, proteins (*e.g.*, IgE or erythropoeitin), peptides and other known drugs. Examples of synthetic functional group components include, but are not limited to, peptide mimetics, functional groups which are synthesized by combinatorial chemistry techniques or rational drug design techniques. Specific types of functional group components which can be used in accordance with the methods of the present invention are discussed in detail below under the heading "*Types of Functional Groups*".

GROUPS A

The groups A of the polyvalent presenter include groups capable of being polyvalently presented in a functional manner, e.g., for treating a disease or condition, and

being on, e.g., attached to, a framework. The groups A can be the same or different. The language "a plurality of groups A" is intended to cover more than one group A wherein each group A within the plurality is independently the same or different. The groups A can be the same or different within categories of types of groups A, e.g., one group A can be a carbohydrate and another group A can be an antibiotic. The groups A also can be different within the same type or category of group A, e.g. two different carbohydrate group A's. In the case of a "homomeric presenter" the A groups presented are the same and in the case of a "heteromeric presenter" the A groups presented are different, e.g., from different categories or different within the same category. For ease of description below and in the claims, the nomenclature A_1 to A_n will be used to denote various members of a plurality of groups A.

As used herein the "groups A" presented by the subject presenters are functional. The groups A also provide their function when attached to or on a framework of a polyvalent presenter. As discussed above and reiterated here, this differs from the art-recognized drug delivery systems, e.g. polymeric drug delivery systems, which release their therapeutic agents and wherein the therapeutic agents provide a function in released form. For example, in preferred embodiments, groups A provide function by biospecifically interacting with eleven or more, i.e., a "plurality of" binding sites B, e.g., to directly provide a therapeutic effect. In certain embodiments, e.g., when the polyvalent presenter is a heteromeric presenter, certain of the groups (e.g., A_n) may not interact with B, but instead provide another function. For example, A_n may influence the interaction of A_1 to binding site B, and thus function as an enhancer group. In other embodiments A_n can be a functional auxiliary group which is capable of influencing the physical characteristics of the polyvalent presenter, e.g., solubility or ability to cross cell membranes. In other embodiments, groups A can provide function by allowing for tracking of the subject presenters, for example, provide a label which can be detected, e.g., a fluorescent or radioactive tag.

The groups A of the present invention can be either synthetic or natural. In addition, groups A can be categorized based on physical characteristics, e.g., molecular size, e.g., groups A can be of low, medium, or high molecular weight. Examples of natural groups A include naturally occurring sugars, proteins (e.g., IgE, or erythropoeitin), peptides, or other known drugs. Examples of synthetic groups include, e.g., peptide mimetics, groups that are synthesized by combinatorial chemistry, or generated through rational drug design. In preferred embodiments, the groups A are not selected from the group consisting of carbohydrate, a polymyxin, e.g., polymyxin B, beta-lactam, a furan-derived compound, a pyran-derived compound, and antibody, alone or in combination with any other member of the group. In even other embodiments, the groups A are not derivatives or fragments of the aforementioned groups A. Specific types of groups A are discussed in detail below under the heading "*Types of Groups A*".

Number of groups A per presenter

Various physical properties of the framework can influence the ability of polyvalent presenters to modulate interactions between groups A and binding sites B. By varying the number of group equivalents, the effectiveness of polymeric presenters can be altered, i.e., can be made to either increase or decrease. For example, large number of attachment points can cause the presenter backbone to collapse onto the surface on which binding sites B are displayed and become less effective in steric stabilization but more effective in competitive Blocking of target sites and/or agonism/antagonism of biological responses.

A systematic study of the effects of substituents on a parent polyacrylamide polymer with 0.2 equivalents of sialic acid (SA) indicated that the net charge, size and hydrophobicity of backbone substituents can effect the success of biological function by presenters (Mammen *et al.* 1995. *J. Med. Chem.* 38:4179). In general, changing the nature of the backbone substituents will affect both affinity and steric stabilization. Moreover, the effectiveness of polymeric inhibitors decreases with increasing charge and size of substituents. Coulombic and steric interactions cause the chains to become more extended and less effective in steric stabilization.

Heteromeric presenters which present multiple, different groups.

Heteromeric presentation can be used to provide both greater *strength* and *specificity* than equivalent monovalent interactions. For example, by presenting an additional type of group (e.g., A_2), the total number of interactions is increased, then the total *strength* of the interaction may increase. The *specificity* of the interaction can increase by differentially regulating the number of groups of A_1 and A_2 that are presented. For example, a presenter bearing both A_1 and A_2 may interact more tightly with to B_1 and B_2 than a presenter bearing A_1 alone or A_2 alone.

In addition to the use of different A groups which impart unique pharmacologic properties to the subject presenters, the use of multiple, different groups A can offer protection against a range of different pathogens.

For example, in one embodiment a polyvalent presenter may present groups A_1 which interact with receptors on the surface of pathogens and A_2 which interact with a second pathogen. In another exemplary embodiment B_1 and B_2 are on the same pathogen.

Types of groups A

The groups A of the present invention include groups useful for treating a disease or condition when presented in a polyvalent manner. These groups can be known groups or drugs or can be novel groups or drugs selected after studying a polyvalent interaction involved in a particular disease or condition. The present invention provides for

the use of known groups A which have been described, (Kiessling, L. L.; Pohl, N. L. *Chem. & Biol.* 1996, *3*, 71-77) and combinatorial methods of developing new groups, and of enhancing the effectiveness of known monomeric groups by presentation in polyvalent form. It is recognized that some applications for the polyvalent presenters may be more favored than others due to more facile access to the site of action. It should be understood that less facile access may be addressed by more direct administration to the site of action.

In certain embodiments, groups known to be involved in cell-pathogen interaction, cell-cell interaction, pathogen-extracellular matrix interaction, and pathogen-pathogen interaction can be presented in a polyvalent manner on the subject presenters.

For example, as shown in the appended example and described in the Appendices, one exemplary group is N-Acetyl Neuraminic acid (a sialic acid), which is the natural binding site for influenza hemagglutinin. The interaction between this sugar and its lectin is the first essential step in influenza viral attachment to its target cell. A very large number of polyvalent sugars are possible groups, including Neu5Ac(2,6)Galactose, Neu5Ac(2,6)Lactose, NeuAc(a2,3)Gal(b1,3)GalNAc, heparin sulfate, and galatosyl ceramide, which have all been shown to be important in the attachment of different viral particles to host cells.

In other embodiments, groups known to modulate other diseases or conditions involving polyvalency can be employed on the subject presenters. For example, groups A can be selected for their ability to mediate the polyvalent interactions between platelets in situations where it is desirable to treat thrombosis.

In preferred embodiments, the subject presenters can comprise known drugs or compounds which have been shown not to be significantly effective in treating a disease or condition when administered in their monovalent form. In these embodiments, the drug becomes significantly effective when presented polyvalently in accordance with the present invention. Therefore, the present invention provides for numerous, existing drugs and non-drug groups that can be incorporated into the subject presenters as Groups A. It will be understood by one of ordinary skill in the art that groups A may have more than one biological effect, and/or may be useful in the treatment of more than one type of disease or condition. It also should be understood that some groups A will be useful for treating diseases or states involving a polyvalent interaction and/or some groups A will be useful for treating diseases or states not previously identified as involving polyvalent interactions. The term "drug" is used below to refer to possible groups A for ease of discussion.

For example, drugs that have effects in the central nervous system are provided for. The treatment of Alzheimer's disease can employ, e.g., tacrine or donepezil as groups A. In another embodiment treatment of alcohol dependence can employ disulfiram as groups A. Treatment of acute and/or chronic pain and/or inflammation can employ analgesics as groups A (e.g., acetaminophen, aspirin, ibuprofen, naproxen, pentazocine,

indomethacin, or diflunisal) or anesthetics (e.g., ropiacaine or remifentanil). Treatment of pain and/or narcotic dependence can employ, e.g., hydrocodone, propoxyphenemeperidine, hydromorphone, morphine, methadone, or oxycodone as groups A. The use of anesthetics is also provided for, such as: epinephrine, xylocaine, mepivacaine, methohexital, bupivacaine, and novocaine. The subject polyvalent presenters can also use cholinesterase inhibitors as groups A, such as, for example, pyridostigmine or neostigmine. Hypnotics can also be incorporated into the subject presenters. For example, fluazepam, pentobarbital, triazolam, temazepam, or secobarbital could be used. Groups A can also be antitussives, for example, pseudoephedrine or codeine. The present invention also provides for the use of anti-migraines as groups A, such as ergot derivatives (e.g., ergotamine or methysergide) or sometheptene, or serotonin (5-HT) antagonists e.g., sumitriptan. Groups A can also be motion sickness remedies, such as meclizine or scopalamine.

In other applications, groups A can also be muscle relaxants, such as: pyridostigmine, neostigmine, succinylcholine, mivacurium, doxacurium, rocuronium, vecuronium, dantrolene, cyclobenzaprine, baclofen, chlorzoxazone, methocarbamol, or papaverine. Nausea can be treated with the use of, e.g., prochlorperazine, chlorpromazine, trimethobenzaminde, perphenazine, hydroxyzine, or ondansetron. Parasympatholytics can also be used as groups A, such as biperiden, phenobarbital, ergotamine, dicyclomine, hyoscyamine, glycopyrrolate. Likewise, the parasympathomimetics, e.g., tacrine, pilocarpine, bethaneechol, edrophonium, yohimbine can be used. Groups A can also comprise parinsonism drugs, e.g., trihexyphenidyl, bentropine, procyclidine, levodopa, bromocriptine, carbidopa, amantadine. The invention also provides for the use of psychotropics in the subject presenters. Likewise, antianxiety agents (e.g., lorazepam, buspirone, chlordiazepoxide, meprobamate, clorazepate, diazepam, and alprazolam) can be incorporated in the subject presenters. Antidepressants, e.g., phenelzine, tranylcypromine, parozetine, fluoxetine, sertraline, amitriptyline, nortriptyline, imipramine, or protriptyline can be used. Antipsychotics (e.g., clozapine, prochlorperazine, haloperidol, loxipine, thioridazine, flupenazine, risperidone, mesoridazine, trifluoperazine, olanzapine, or chlorpromazine) can also be used as groups A. The use of psychostimulants (e.g., pemoline or methylphenidate) is also provided for. Sedatives (e.g., mephobarbital, secobarbital, or temazepam) can also be used as groups A. Seizure disorders can be treated by polyvalent presenters bearing, e.g., felbatol, gabapentin, phenytoin, mephenytoin, ethotoin, lamotrigine, methsuximide, phensuximideethosuximide, cabamozepine, phenacemide, or cabamazepine. Sympatholytics such as, e.g., phentolamine can also be used. Groups A can also include, e.g., anticonvulsants (e.g., fosphenytoin), antidepressants (e.g., mirtazapine) groups which can be used to treat multiple sclerosis (e.g., glatiramer) or epilepsy (e.g., topiranate), or groups which can be used to inhibit angiogenesis (e.g., art-recognized angiogenesis inhibitors).

The use of groups A with effects in the cardiovascular system is also provided for. For example adrenergic agents, such as doxazosin, terazosin, prazosin, methyldopate, clonidine, labetalol. In another embodiment, angiotensin converting enzyme inhibitors such as captopril, lisinopril, tradolapril, or enalapril can be used. Polyvalent presenters can also be made to present angiotensin II receptor antagonists, such as losartan or valsartan. The use of antiarrhythmics is also provided for; examples include: disopyramide, procainamide, quinidine, propafenone, flecainide, tocainide, propanolol, sotalol, amiodarone, digoxin. In other embodiments, β-Blockers such as timolol, metoprolol, and atenolol can be used as groups A. In a further embodiment, calcium channel clockers can be employed as presented groups. Examples include nifedipine, nicardipine, diltiazam, felodipine, and verapamil.

Diuretics (e.g., acetazolamide, ethacrynic acid, furosemide, spironolactone, amiloride, chlorothiazide, and hydrochlorothiazide) can also be used as groups A. Vasodilators (e.g., papaverine, hydralazine, and amrinone) can also be employed in the subject presenters. The use of vasopressors (e.g., metaraminol, phenylephrine, or isoproterenol) is also provided for. In another embodiments groups A can comprise e.g., mecamylamine. Groups A can be a hypolipidemic, e.g., clofibrate, gemfibrozil, simvastatin, lovastatin, simvastatin, atorvastatine, or niacin. Deep vein thrombosis can be treated by the use of e.g., danaparoid as groups A. In a further embodiment, antihypertensives, such as midodrine can be used.

For the treatment of cancer, groups A can include, for example, anti-androgens (e.g., leuprolide or flutamide), cytocidal agents (e.g., adriamycin, doxorubicin, taxol, cyclophosphamide, busulfan, cisplatin, α2-interferon) anti-estrogens (e.g., tamoxifen), antimetabolites (e.g., fluorouracil, methotrexate, mercaptopurine, thioguanine). Groups A can also comprise hormones (e.g., medroxyprogesterone, estradiol, leuprolide, megestrol, octreotide, or somatostatin). In still other embodiments, groups A can include, e.g., irinotecan, gemcitabine, toptecan, nilandrone, or docetaxel.

In other embodiments, the subject presenters can be used in gastrointestinal applications. For example, antispasmodics or anticholinergics can also be included as groups A (e.g., dicyclomine, hyoscyamine, glycopyrraolate, trihexyphenidyl or atropine). Appetite suppressants can also be used as groups A (e.g., dextroamphetamine, benzphetamine, phentermine, and ormazindole. In other embodiments, groups A can also include anti-diarrhea agents (e.g., loperamide, diphenoxylate, octreotide. The subject presenters can also include, e.g., proton pump inhibitors (e.g., lansoprazole or omeprazole) or vasodilator, e.g., opioids.

Groups A that modulate the endocrine system can also be polyvalently presented. For example, contraceptives (e.g., ethinodiol, ethinyl estradiol, norethindrone, mestranol, desogestrel, medroxyprogesterone). Groups A that modulate diabetes can also be used (e.g., glyburide or chlorpropamide). Anabolics, such as, testolactone or stanozolol can

also be incorporated into the subject presenters. Androgens (e.g., methyltestosterone, testosterone or fluoxymesterone) can also be used as groups A. Antidiuretics (e.g., desmopressin) can also be used as groups A. The subject presenters can also display e.g., calcitonins in a polyvalent manner. Estrogens (e.g., diethylstilbesterol) can also be employed. Groups A can also be glucocorticoids (e.g., triamcinolone, betamethasone). The use of progestogens as groups A is also provided for, such as, norethindrone, ethynodiol, norethindrone, levonorgestrel, and ethinylestradiol. In yet other embodiments thyroid agents (e.g., liothyronine or levothyroxine) or anti-thyroid agents (e.g., methimazole) can be used. In other embodiments, hyperprolactinemic disorders can be treated using e.g., cabergoline as groups A. In still other embodiments, diabetes can be treated, using e.g., miglitol or insulin lispro). The use of hormone suppressors (e.g., danazol or goserelin) is also provided for. In other embodiments, groups A can include oxytocics (e.g., methylergonovine or oxytocin). Prostaglandins can also be employed as groups A, e.g., mioprostol, alprostadil, or dinoprostone.

The subject polyvalent presenters can also be used in dermatological applications. Exemplary groups A with dermatological effects can include, e.g., anti-acne agents (such as, isotretinoin, adapalene or tretinoin). Other groups A can be employed for dermatological applications, e.g., for the treatment of pruritis, include, e.g., alclometasone, benzocaine, hydroxyzine, fluticazone, mometasone, fluocinolone, clobestasol, or desoximetasone.

Groups A can also affect clotting. For example, poly (A) can comprise the anticoagulant, heparin. In other embodiments groups A can comprise e.g., antithrombin III, or a platelet inhibitor, such as abciximab and/or ticlopidine.

In other embodiments, e.g., the groups A may be chosen for their ability to affect immunomodulation. For example, the release of histamine from mast cells and basophils involves the polyvalent interaction between an allergen and IgE receptors on the cell surface. In such instances groups A may comprise, e.g., antihistamines, such as benadryl, loratadine, brompheniramine, periactin, promethazine, terfenadine, fexofenadine, azelastine and/or clemastine. In still another embodiment groups A can comprise mast cell stabilizers, e.g., lodoxamide and/or cromolyn.

Other groups A which modulate the immune system can include: e.g., steroids (triamcinolone, beclomethazone, cortisone, dexamethasone, prednisolone, methylprednisolone, beclomethasone, or clobetasol), histamine H_2 antagonists (e.g., famotidine, cimetidine, ranitidine), immunosuppressants (azathioprine, cyclosporin). Groups with anti-inflammatory activity, such as, for example, sulindac, etodolac, ketoprofen,

ketorolac can also be used as groups A. Antihistamines (e.g., fexofenadine or azelastine) can also be employed.

Groups A that modulate the respiratory system can also be used. For example, mucolytics (e.g., guaifenesin), Anti-inflammatory agents (e.g., cromolyn, flunisolide, beclomethasone, or budesonide) can also be used in the subject presenters. The use of bronchodilators, e.g., ipratropium, metaproterenol, terbutaline, isoetharine, metaproterenol, albuterol, or theophylline as groups A is also provided for. For the treatment of asthma agents such as, e.g., zafirlukast or zileuton can be employed.

Groups A can comprise antimicrobial agents such as, e.g., quinolones (e.g., ciprofloxacin, norfloxacin, ofloxacin, enoxacin, lomefloxacin), sulfonamides (sulfasalazine or sulfamethoxazole) or, for example, polymyxin B, bacitracin, neomycin, oxytetracycline, tobramycin, sulfacetamide, fosfomycin, and penicillin, antivirals (e.g., trifluridine, zidovudine (AZT), zalcitabine (ddV), didanosine (ddl), stavudine (d4T) or other reverse transcriptase inhibitors, e.g., sold by Merck, protease inhibitors (e.g., saquinavir, indinavir, ritonavir), acyclovir, famciclvir, ribavirin, zidovudine, nevirapine, cidofovir, or penciclovir) antiparasitic agents (e.g., thiabendazole, chloroquine, sulfadoxine, pyrimethamine, mefloquine, metronidazole, albendazole, or ivermectin), or antifungals (e.g., butenafine, butoconazole, terconazole, tioconazole, clotrimazole, or miconazole). In certain instances it will be obvious to the ordinarily skilled artisan that the use of particular antimicrobials is preferred for treating infections as particular sites, e.g., ophthalmic infections.

In other embodiments, the penicillin adjuvant, probenecid, can also be employed as groups A.

Urinary tract agents for use as groups A can include, e.g., uricosuric agents, e.g., sulfinpyrazone. Antimicrobials, e.g., indanyl carbenicillin, nitrofurantoin, nalidixic acid, neomycin, bacitracin, or polymyxin B can also be used as groups A. The subject presenters can also present antispasmodics (e.g., oxybutynin or flavoxate). Calcium oxalate stone preventatives can also be used as groups A, e.g., allopurinol). Prostatic hypertrophy modifiers (e.g., terazosin or finasteride) can also be employed. The subject presenters can also be used in the treatment of cystitis, using e.g., pentosan as groups A.

The subject presenters are also useful in ophthalmic applications. For example, β-Blockers (e.g., brominide or betaxolol), anti-inflammatories (e.g., clopatadine), or drugs useful in the treatment of glaucoma (e.g., latanoprost) can be used as groups A. The invention also provides for the use of carbonic anhydrase inhibitors, e.g., dichlorphenamide, methazolamide, or dorxolamide as groups A.

For dental applications e.g., aphthous ulcers, groups A can include, for example, amlexanox.

In other embodiments, the groups A can be independently selected from erythropoeitin, tacrolimus, human growth hormone, tissue plasmin activating factor, platelet activating factor, interleukin stimulating factors, granulocyte stimulating factors, macrophage stimulating factors, small molecules secretagogues which target the receptors of protein products such as growth hormone secretagogues, EPO secretagogues, and insulin secretagogues.

Some particular examples of polyvalent presenters, by way of illustration and not limitation, include polyvalent sialic acid against HA on flu virus, two derivatives of Sialyl Le^x with platelet containing P-selectin, polyvalent Sialyl Le^x against endothelium containing both E and P selectins, barbiturate (benzodiazepine) binding the $GABA_B$ complex receptor containing two different receptor sites, RGD and Sialyl Le^x binding leukocyte having both integrins and selectins, and C_{5A} and Gal-X-Gal binding tumor (which binds gal-x-gal) and also proteins of complement cascade.

Ancillary Groups

As used herein, an "ancillary group" is a moiety which alters a characteristic of the polyvalent presenter and/or the components making up the polyvalent presenter (*e.g.*, the framework component, the functional group component, the spacer group, *etc.*). Properties which can be modified include, for example, solubility (in water, fats, lipids, biological fluids, *etc.*), hydrophobicity, hydrophilicity, framework flexibility, antigenicity, molecular size, molecular weight, *in vivo* half-life, *in vivo* distribution, biocompatibility, immunogenicity, stability, strength of binding to the polyvalent target, *etc.*

Those of skill in the art will understand that there is substantial, but not complete, overlap between many of these characteristics and the ancillary groups which will effect changes in these characteristics. For example, it is expected that the introduction of one or more poly(ethylene glycol) (PEG) groups onto the framework of a polyvalent presenter will enhance hydrophilicity and water solubility, increase both molecular weight and molecular size and, depending on the nature of the unPEGylated framework, may increase the *in vivo* retention time. Further, it is expected that PEG will decrease antigenicity and may, through hydrogen bonding to solvent molecules (*e.g.*, water), enhance the overall rigidity of the polymeric presenter. Similar areas of overlap between framework

characteristics and ancillary groups capable of affecting these characteristics will be apparent to those of skill in the art.

Ancillary groups which enhance the water solubility/hydrophilicity of the polyvalent presenter are useful in practicing the present invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, poly(ethylene glycol), alcohols, polyols (*e.g.*, glycerin, glycerol propoxylate, saccharides, including mono-, oligo- and polysaccharides, *etc.*), carboxylate, polycarboxylates (*e.g.*, polyglutamic acid, polyacrylic acid, *etc.*), amines, polyamines (*e.g.*, polyglycine, poly(ethyleneimine, *etc.*) to enhance the water solubility and/or hydrophilicity of the polyvalent presenter. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyol. In particularly preferred embodiments, the ancillary group will be a poly(ethylene glycol).

The incorporation of lipophilic ancillary groups, within the structure of the polyvalent presenter, to enhance the lipophilicity and/or hydrophobicity of the presenter is within the scope of the present invention. Lipophilic groups of use in practicing the instant invention include, but are not limited to, aromatic groups and polycyclic aromatic groups. As used herein the term "aromatic groups" incorporates both aromatic hydrocarbons and heterocyclic aromatics. The aromatic groups may be either unsubstituted or substituted with other groups, but are at least substituted with a group that allows their covalent attachment to the polyvalent presenter. Other groups of use in practicing the instant invention include fatty acid derivatives that do not form bilayers in aqueous medium.

In preferred embodiments, the lipophilic ancillary group will be a cyclic group such as a hydrocarbon or heterocycle. In other preferred embodiments, the cyclic group will be a six-membered ring or two or more fused six-membered rings. In particularly preferred embodiments, the ancillary group will be a phenyl or a naphthyl group.

Also within the scope of the present invention is the use of ancillary groups that result in the polyvalent presenter being incorporated into a vesicle such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphate, carboxylic, sulfate, amino, sulfhydryl, nitro, and other like groups. Hydrophobicity could be conferred by the inclusion of groups that include, but are

not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups and such groups substituted by one or more aromatic, cycloaliphatic or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or dilinoleoylphosphatidylcholine could be used. Other compounds lacking in phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the polyvalent presenter framework can be manipulated by the inclusion of ancillary groups that are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the framework or bonds between the framework and the ancillary group(s) or bonds between the framework and the functional groups. Rigid groups can include, for example, those groups whose conformational lability is restrained by the presence of rings and/or multiple bonds. Other groups that can impart rigidity include polymeric groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either negatively or positively charged, the similarly charged ancillary groups will force the presenter framework into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other will tend to hold the framework in a configuration that maintains the separation between the like-charged ancillary groups. Further, ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and will enter into both inter- and intramolecular ionic bonds. This non-covalent bonding mechanism will tend to hold the framework into a conformation that allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, bear a latent charge which is unmasked, following addition to the framework, by deprotection, a change in pH, oxidation, reduction or other mechanisms known to those of skill in the art, is within the scope of the present invention.

Bulky groups can include, for example, large atoms or ions (*e.g.*, iodine, sulfur, metal ions, *etc.*) groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon multiple bonds (*i.e.*, alkenes and alkynes). Bulky groups can also include oligomers and polymers that are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain polymers.

In preferred embodiments, rigidity is imparted by the presence of cyclic groups (*e.g.*, cyclic hydrocarbons, heterocycles, *etc.*). In other preferred embodiments, the ring is a six-membered ring. In still further preferred embodiments, the ring is an aromatic group such as, for example, phenyl or naphthyl.

Altering the antigenicity of the polyvalent presenter by judicious choice of ancillary group(s) is within the scope of the present invention. In certain applications, the antigenicity of the polyvalent presenter may need to be decreased. As discussed above, masking groups such as, for example, poly(ethylene glycol) are known in the art to have the capacity to lower the antigenicity of both monovalent and polyvalent compounds. In other applications, it may be desirable to enhance the antigenicity of the polyvalent presenter and thus, elicit an immune response. In these applications, the ancillary group may comprise a group known in the art to enhance the immunogenicity of a hapten. Groups appropriate for enhancing the immunogenicity of a polyvalent presenter include, but are not limited to, proteins such as keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other groups capable of enhancing the antigenicity of a polyvalent presenter will be known to those of skill in the art.

The *in vivo* half-life and *in vivo* distribution are functions of numerous molecular properties including molecular size, molecular weight, charge, hydrophobicity/hydrophilicity, antigenicity, biodegradability and the presence or absence of targeting groups on the polyvalent presenter. The term "targeting groups," as used herein, refers to groups that have an affinity for a cellular receptor. Methods of altering these properties to achieve desired changes in the half-life or distribution of compounds administered *in vivo* are well known in the pharmaceutical and medicinal chemistry arts.

Approaches for Novel group A Identification.

In addition, the present invention provides for the synthesis of oligosaccharide groups for use as group A (Horton, D. *Advances in Carbohydrate Chemistry and Biochemistry;* Academic Press: San Diego, 1995). Protein analogs can be synthesized (Maassen, J. A.; Terhorst, C. Eur. J. *Biochem* 1981,115, 153-158; Wang, K. S., *et al., J. Virol. 1992, 66,* 4992-5001; Mastromarino, P., *et al.,* J. *Gen. Virol.* 1987, *68,* 2359-2369). Computer based design tools can be also used to synthesize groups A (Jorgensen, W. L. *Chemtracts: Org. Chem.* 1995, 8, 374-376).

The invention also provides methods for discovery of new groups A within the context of polyvalency. For example, a single phage in a phage-display library, single pins in a spatially addressed combinatorial array, or single beads in a combinatorial library produced by the mix-and-split method, each bear only one type of group, which is presented in polyvalent form. Thus, libraries can be screened directly in bound form against polyvalently presented B for activities such as simple adhesion, or adhesion which leads to a specific result, such as infection, cell death, cell proliferation, morphological change, or production of an easily detectable reporter such as green fluorescent protein. A typical resin bead can have a loading of ~100 pmol, or about 60 trillion copies. Not all of these copies will reside at the surface of all solid supports, but supports can be chosen for e.g., porosity or surface functionalization. In contrast, phage can display 3-1000 copies of peptide groups on the surface, depending on the coat protein to which the library is fused. Any species of group A identified as a candidate worthy of further analysis could be subsequently tested in soluble, polymeric form.

By way of example, beads in a library to which bacterial, fungal, or neutrophil cells specifically adhere carry groups that may serve as useful groups for a polyvalent presenter of the present invention. Libraries of ~ 1,000,000 different peptides can be prepared by split synthesis, and diverse libraries of other types of compounds may be obtained. The structures of selected groups could be determined from the synthesis history or could be obtained by sequencing, mass spectrometry, deconvolution, or encoding.

For example, phage-displayed peptide libraries can be used (see e.g., Doyle, M.V., *et al.*, "The Utilization of Platelets and Whole Cells for the Selection of Peptide Ligands from Phage Display Libraries". In "Combinatorial Libraries: Synthesis, Screening, and Application Potential". Cortese, R., Ed.; Walter de Gruyter: Berlin; 1996; pp. 159-174; Fong *et al.* Drug Dev. Res. 1994, 33, 64-70; Goodson, *et al.*, Proc. Natl. Acad. Sci. U.S.A. 1994, 91, 7129-7133). In one example, PIII-modified phage (3-5 copies per particle) can be selected ("panned") for interaction with whole, activated platelets, leading to the identification of five different classes of platelet-binding peptide groups. Synthetic peptides derived from phage sequences have been tested in inhibition of phage-platelet interactions and to prevent platelet aggregation, although the observed IC50 values were low.

Combinatorial synthesis of polysaccharides using highly efficient glycosyl donors has also been described. (Liang, *et al.*, Science, 274:1520, 1996). Such a strategy depends on anomeric sulfoxides as glycosyl donors, which are highly reactive and allow synthesis of polysaccharides in very high yield, which is an essential characteristic of combinatorial libraries in general. Libraries can be created on beads and screened for interaction to polyvalent lectins in solution. Adhesion assays or agglutination assays can be used in this system.

Techniques of rational drug design, including the use of computer-based design tools (Jorgensen, W. L. "A new method for predicting binding affinity in computer-aided drug design "*Chemtracts: Org. Chem.* 1995, 8, 374-376) can be used to identify novel groups A for polyvalent presentation.

GROUPS A -ATTACHMENT

The groups A can be attached to or formed as part of a framework using a means which allows for polyvalent presentation of the groups. The attachment can be direct, e.g., without a linker, or can be indirect in that the groups A are attached using linkers. The groups A also can be attached to monomer units before polymerization or after polymerization. The order of the attachment steps also is not critical to the invention as long as the formed presenter displays the groups A in a polyvalent manner. For example, the group A can be attached first to a linker and then the linker-group A moiety can subsequently be attached to a framework or vice versa. Alternatively, the linker can be attached to the framework and then the groups A can be attached to the framework-linker moiety. Groups A also can be incorporated into the polyvalent presenters using bifunctionalized monomers bearing groups A, which can then be polymerized. Alternatively, a polymer can be prepared that is reactive, i.e., activated for coupling and then groups A can be caused to react with the polymer (a "preactivation" strategy).

A preferred embodiment involves attaching a linker to the groups A. As discussed above, the linker includes moieties capable of positioning or attaching a group A on a framework. In an even more preferred embodiment, the linker is a moiety independent of the group A and the framework. An example of a preferred type of linker is a linker that leaves a primary amine at the untethered end of the linker. The modified group can then be attached to an activated carboxylic acid on the polymer, forming an amide linkage. Amide bonds are relatively stable, occur widely in natural systems, and can be formed cleanly by a range of well-developed methods. Additional advantages can be obtained using a particular linker.

Cationic polymers tend to be more antigenic than anionic polymers. The cationic modified ligand can be used in limiting quantities to ensure the consumption of all of the modified groups, leaving only excess carboxylic acid groups on the polymer, which

imparts water solubility. As discussed above, the linkers also provide spacing of the group A from the framework. In certain embodiments, the linkers also impart flexibility to the polyvalent presenter, e.g., flexible movement of the group. Exemplary linkers for use in the subject presenters include poly(gly), alkyamines, e.g., ethylamine, hydrocarbon moieties, thioether, poly (ethylimine) and poly(asp) and polyethers such as PEG chains.

The "preactivation" method allows one to construct frameworks, e.g., polymers, with a defined mole fraction of its monomer units presenting different groups. For example, the method can utilize an activated ester of poly(acrylic acid), such as poly(N-acryloylsuccinimide) or pNAS, made from polymerization of N-acryloylsuccinimide. The NHS ester of pNAS in DMF can be reacted sequentially with different primary amines, (e.g., R_1NH_2 and R_2NH_2) to form amide bonds. The remaining, unreacted ester groups can be converted to carboxamides or carboxylic acids by treating with excess NH_3 or OH^-, respectively. The distribution of these groups along the polymer backbone is thought to be random using this method. Furthermore, the degree of polymerization (the total number of monomer units in one polymer) can be held constant as the influence of different side groups on effectiveness is examined. The efficiency of the amide-forming reaction between amines and pNAS can be estimated by use of both 1H-NMR spectroscopy and combustion analysis. This reaction consistently proceeds to > 90% completion.

Another example of direct "copolymerization" is illustrated by the use of a mixture of monomeric derivatives of acrylamides in refluxing THF (Spaltenstein, *et al.*, *supra*). Copolymerization may introduce two major uncontrolled variables in polymerization: (i) unknown differences in rate constants for copolymerization among differently N-substituted acrylamides might result in non-uniform distribution of group along the backbone of the polymer; (ii) the polydispersity, tacticity and length of the polymer might vary as the side chain is altered. This can occur owing to the differences in rates involved in the polymerization process (propagation; termination). The "preactivation" method may be preferable where the pA(R) with uniform structural features are needed.

In preferred embodiments, polymers will be activated using a process that has a high yield and allows for easy removal of by-products from the method. Further considerations include reactivity of by-products with the attached groups, tendency to cause racemization (e.g., with poly(amino acids)).

For example, there is a range of chemical methods for activating carboxylic acids for reaction with amines. The relative reactivity of activated carboxylic acids decreases in this order: $RCOHal > (RCO)_2O + RCON_3 > RCO_2R > RCONH_2 > RCOR$, and

also increases with the nucleophilicity of the attacking amine. In preferred embodiments an internal anhydride is used.

With an anhydride or succinimide unit in the polymer backbone, an amide bond is readily formed when reacted with an amine. Intramolecular anhydrides in peptides also can be formed using α-amino-N-carboxylic anhydrides or thiocarboxylic anhydrides.

In certain embodiments mixed anhydrides can be used, e.g., carbodiimides and others. Common approaches to forming mixed anhydrides used in peptide chemistry include forming carbodiimides (using, for example, dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylamino) propylcarbodiimide (EDC)). EDC may be useful for water-soluble polymers. Other exemplary anhydrides can be formed from reagents such as chloroformates or quinoline derivatives (EEDQ, IIDQ).

As mentioned above, in other embodiments carboxylic acid azides can be used. In the case of the use of carboxylic acid azides, prior reaction is required to form the azide. In other embodiments, imidazoles, such as carbonyldimidazole can be employed.

In yet other embodiments, compounds, such as p-nitrophenol or N-hydroxysuccinimide, that form activated esters, may also be used.

In still other embodiments the polymers used contain hydroxyl groups, and so methods for derivatization of hydroxyl groups can be employed. Such methods are known to the ordinarily skilled artisan.

Alternatively, combinatorial methods can be used to create polyvalent presenters. Briefly, a quasi-solid-state, combinatorial approach can be used to generate libraries of backbone bearing groups A_1 to A_n. Combinatorial libraries are formed that consist of an array of synthetic polyvalent presenters, wherein the polyvalent presenters in the array differ from one another in terms of their compositions, structures, properties, functions, *etc.* In making the arrays of polyvalent presenters, one can vary, *inter alia*, the chemical structure of the framework component, the chemical structure of the functional group component, the chemical structure of the ancillary group, the chemical structure of the spacer group, the chemical nature of the framework component, the chemical nature of the functional group component, the chemical nature of the ancillary group, the chemical nature of the spacer group, the amount of framework component delivered, the amount of functional group component delivered, the amount of ancillary group component delivered, the amount of spacer group delivered, the number and/or amount of different framework components delivered, the number and/or amount of different functional group components delivered, the number and/or amount of different ancillary groups delivered, the number

and/or amount of different spacer groups delivered, the nature and number of the linkages between the various components (*e.g.*, the nature of the linkages of the spacer group), the reaction parameters (*e.g.*, reactant solvents, reaction temperatures, reaction times, reaction initiators, reaction catalysts, the atmosphere in which the reactions are carried out, the pressure at which the reactions are carried out, the rates at which the reactions are quenched, *etc.*); the stoichiometry of the various components; the order in which the different components are delivered, *etc.*

As such, in one embodiment, the invention provides a method of making an array of polyvalent presenters, the method comprising: (a) delivering a first activated framework component of a first polyvalent presenter and a first activated framework component of a second polyvalent presenter to first and second reaction vessels; and (b) delivering a first functional group component of the first polyvalent presenter and a first functional group component of the second polyvalent presenter to the first and second reaction vessels, thereby forming the array of at least two different polyvalent presenters. The preactivated reactants are allowed to react. This process is optionally repeated, with additional components (*e.g.*, framework components, functional group components, ancillary groups, spacer groups, *etc.*) and/or different reaction parameters (*e.g.*, different reaction temperatures, reaction catalysts, reaction solvents, *etc.*), to form a vast array of polyvalent presenters.

Once formed, the array of polyvalent presenters can be screened/assayed for polyvalent presenters having useful properties. Properties which can be screened for include, but are not limited to, the following: biological activities, binding affinities, biological properties, pharmacological properties, oral bioavailabilities, circulatory half-lives, agonist activities, antagonist activities, solubilities, *etc.* The array of polyvalent presenter can be screened for useful properties sequentially or in parallel. Additionally, the array can be screened *in situ* or, alternatively, the polyvalent presenters can be screened in other than an *in situ* manner (*e.g.*, the polyvalent presenters can be removed from the substrate and then screened). Once identified, the polyvalent presenters having useful properties can be prepared on a large-scale.

Spacer Groups

In another embodiment of the present invention spacer groups or, interchangeably, linker groups are interposed between the framework and the functional group and/or the framework and the ancillary group.

The constructs of this embodiment of the invention have the following general formulae:

$$R^1\{—R^2(—R^3)_n\}_m \quad \text{(II)}$$

$$R^1\{—R^2(—R^3)_n\}_m\{—R^4(—R^5)_s\}_t \quad \text{(III)}$$

The symbol R^1 in both of these formulae represents the multifunctional framework providing a multitude of attachment sites for spacer groups. Polymers, including dendrimers, polypeptides, polysaccharides and others, are generally useful for this framework. With its multitude of attachment sites, the backbone serves an amplifying function for the functional and/or ancillary groups.

In both of these formulae, the symbols R^2 and R^3 represent a spacer group and a functional group, respectively. The symbol m represents the number of functional groups attached to each spacer. The symbol n represents the number of spacers, and their associated functional groups, which are attached to the framework. As explained above, in general, the number of functional groups and the number of ancillary groups and the number of spacers are such as to achieve greater than ten total functional groups and/or ancillary groups on the polyvalent presenter.

In Formula III, the symbols R^4 and R^5 represent a spacer group and an ancillary group, respectively. The symbol s represents the number of ancillary groups attached to each spacer. The symbol t represents the number of spacers, and their associated ancillary groups, which are attached to the framework. In general, the number of functional groups and the number of spacers are such as to achieve greater than ten total functional groups on the polyvalent presenter. For example, "m" may be six and "n" may be 3 so that a total of eighteen functional groups are present.

In those embodiments represented by Formula II, only the spacer/functional group construct is attached to the framework. In embodiments represent by Formula III, both the spacer/functional group and spacer/ancillary group constructs are attached to the framework. In Formula III, the spacers R^2 and R^4 can be either the same group or different

and may be present in an approximately 1:1 molar ratio, or a different molar ratio. In the interest of brevity and simplicity, the following discussion is phrased in terms of R^2, however, it should be understood that both R^2 and R^4 are encompassed within this discussion. Correspondingly, the discussion concerning R^3 (functional groups) also encompasses R^5 (ancillary groups).

The spacer R^2 can be any of a wide variety of molecular structures, and will be at least bifunctional to permit attachment to both R^1 and R^3, optionally through linkage groups. Spacers can be those that are stable or can be cleaved *in vivo* by the biological environment. Spacers with multiple binding functionalities at the terminus (*i.e.*, the R^3 end) accommodate a multitude of functional groups and/or ancillary groups. Spacers of this type serve an amplifying function in a manner similar to that of the framework, although to a lesser extent. Other spacers useful in the invention may have only a single functionality at either end, in which case the value of "n" will be 1 and the value of "m" will be greater than 10 . In addition to the property of *in vivo* cleavability mentioned above, a property of prominent interest is hydrophilicity. Still other useful properties are the ability to lower antigenicity and to increase molecular weight. Spacers with still further properties can be utilized to advantage as well, as will be readily apparent to those skilled in the art.

The functional groups represented by R^3 are as described above for group A. The spacer R^2 may be either a straight-chain or a branched-chain structure. Preferred R^2 groups are those which include a straight chain within their structures, either as the entire spacer group or as the backbone of a branched-chain group. The straight chain may be a chain of carbon atoms or of carbon atoms interrupted with one or more heteroatoms such as oxygen atoms, sulfur atoms or nitrogen atoms. The chain can also be substituted with aromatic groups. The bonds forming the chain may be single bonds, double bonds or triple bonds, although single bonds are preferred. The length of the chain is not critical and may vary widely, depending on the desired relationship between the molecular weight of the construct and the number of functional and/or ancillary groups included on the construct. Best results will generally be obtained with chain lengths ranging from 4 atoms to 1,000 atoms, with preferred chains being those of 6 atoms to 100 atoms, and the most preferred being those of from 10 atoms to 50 atoms. The chain as thus described is the backbone of the spacer itself, and does not include atoms, groups or side chains bonded to the serially

bonded atoms forming the backbone. It does, however, include linking groups at the chain termini joining the chain to R^1 and R^3, when such linking groups are present.

In certain embodiments of the invention, the spacer will be hydrophilic in character to impart hydrophilicity to the construct. The spacer may thus be any hydrophilic group among those known in the art. Examples are polyalkylene glycols, optionally substituted with groups that may or may not add to their hydrophilic character. Among polyalkylene glycols, polyethylene glycol is a preferred example. Examples of the optional substitutions are alkyl groups, alkoxy groups and hydroxy groups. Unsubstituted polyethylene glycol is particularly preferred. The length of the optionally substituted polyalkylene glycol is not critical and may vary. Selection of the length will be governed by such considerations as achieving the desired molecular weight for the construct and imparting the desired degree of hydrophilic character. In most applications, polyalkylenes having molecular weights ranging from about 100 daltons to about 20,000 daltons will provide the best results, with a range of from about 200 daltons to about 1,000 daltons preferred.

In embodiments of the invention in which the spacer provides *in vivo* cleavability to the construct, the spacer may contain any of a variety of groups as part of its chain which will cleave in a biological fluid at a rate which is enhanced relative to that of constructs which lack such groups. Accelerated rates of cleavage enhance the rates of removal of the framework from the functional and/or ancillary groups of the polyvalent presenter. Such removal can be used to reduce toxicity or enhance activity of the functional groups. While the degree of cleavage rate enhancement is not critical to the invention, preferred examples of these spacers are those in which at least about 10% of the cleavable groups are cleaved in the biological fluid within 24 hours of administration, most preferably at least about 35%. Preferred cleavable groups are ester linkages and disulfide linkages.

In further embodiments of the invention, the spacer both imparts a hydrophilic character to the construct and includes a cleavable group as referred to above.

Structural formulas for the spacer vary widely. The discussion below is offered only as an example of one type of spacer useful in practicing the instant invention and is not intended to serve as a limitation on the types of spacers of use in practicing the instant invention. One group of structural formulas for spacers which impart a hydrophilic character

to the construct are those in which m of Formulae II and III above is 1, and R^2 of Formulae II and III is represented by either of Formulas IV, V or VI below:

$$X—R^6—Y—R^7—Z \quad (IV)$$

$$X—R^7—Y—R^6—Z \quad (V)$$

$$X—R^6—Z \quad (VI)$$

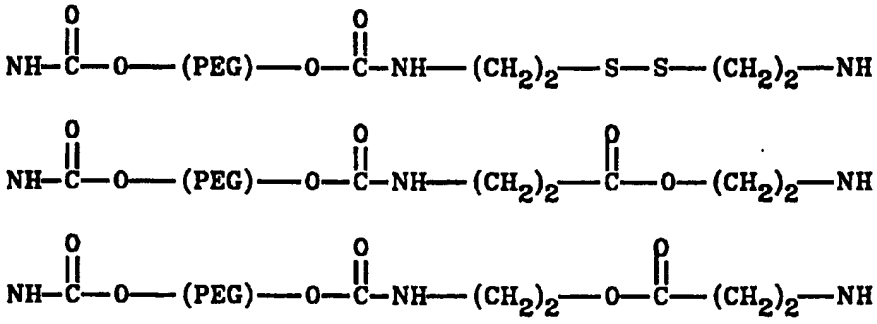
In each of these formulas, the hydrophilic component is represented by R^6, which is a polyethylene glycol group having a formula weight of about 100 daltons to about 20,000 daltons, preferably from about 200 daltons to about 1,000 daltons.

In Formulas IV and V, the group R^7 represents a cleavable group that increases the rate of cleavage of the construct in blood. The group is either a disulfide group S—S, or an ester group oriented in either direction, *i.e.*, C(O) —O or O—C(O). Upon cleavage of constructs in which R^2 is represented by Formula IV, the polyethylene glycol group will remain with the polymeric framework R^1. Conversely, upon cleavage of constructs in which R^2 is represented by Formula V, the polyethylene glycol group will remain with the functional and/or ancillary group.

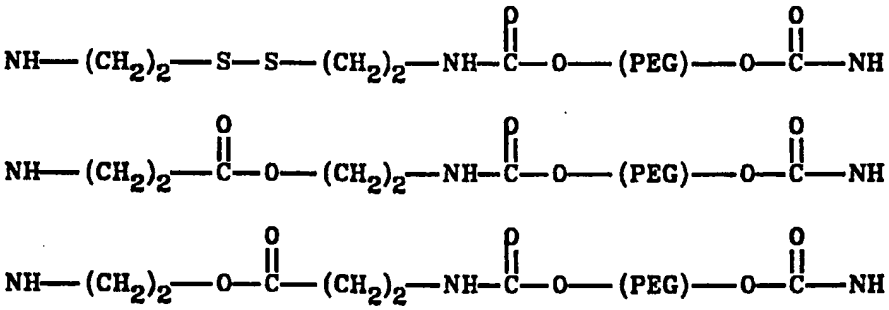
The symbols X, Y and Z represent inert linking groups which serve to join the R-groups together. The nature of these linking groups is not critical, and their selection will be largely a matter of convenience as determined by the means of synthesis of the construct. The term "inert" in this context means essentially non-toxic, non-immunogenic, and stable with respect to cleavage or dissociation over the typical period of time required for use of the construct in a clinical or diagnostic procedure. Examples of inert linking groups useful for this purpose are alkylamino or aminoalkyl groups such as $(CH_2)_q$—NH and NH—$(CH_2)_q$, carbamoyl groups such as NH—C(O) —O and O—C(O) —NH, and alkylcarbamoyl or carbanoylalkyl groups such as $(CH_2)_q$—NH—C(O) —O and O—C(O) —NH— $(CH_2)_q$. The symbol q in these groups may vary, but in most cases will generally range from 1 to 10, with 2 to 4 preferred, and 2 particularly preferred. In the context of this invention, these groups may be defined such that terminal atoms in X or Z may be native to R^1 or R^3, respectively. For example, a terminal

NH group in the definition of X or Z may be formed from an amino functional group on R^1 or R^3 or other N-bearing group which can react to form the NH of the linking group. The same is true of a terminal O atom.

Examples of structures defined by Formula IV are as follows:



Examples of structures defined by Formula V are as follows:



In each of the above six structures, the symbol "PEG" refers to a polyethylene glycol segment with terminal hydroxyl groups removed, thereby terminating in ethylene groups at both ends. Spacer groups of a similar general motif that do not contain PEG will also be useful in practicing the instant invention.

As a variation of Formula IV, a structural formula for a spacer that supports eleven or more functional groups is represented by Formula VII:

$$X—R^6—Y'(—R^7—Z)_r \qquad (VII)$$

In this formula, R^6, R^7, and X are as defined above, with Z restricted to $(CH_2)_q$—NH. The symbol Y' represents a group of the formula

$$O—C(O)—NH—(CH_2)_q—CH_3 \qquad (VIII)$$

in which q is 1 to 3, and two or more of the H atoms bonded to the C atoms in the $(CH_2)_q$—CH_3 portion of the formula are replaced by the substituent NH— $(CH_2)_s$—NH where s is 2 to 4, the number of such substituents being determined as mentioned above for "m" and "n" of Formulae II and III. The result is that the spacer is a branched structure containing two or more reactive NH groups for attachment of functional and/or ancillary groups.

The symbol r of Formula VII is either zero or a number equal to n. When r is zero, the spacer lacks a cleavable group, whereas when r is other than zero, a cleavable group is included for each functional NH group on the Z linker to which a functional group or ancillary group is attached.

In preferred examples of Formula VIII, q is 2 to 6, and in most preferred examples q is 2 or 3.

As a further variation, the terminal CH_3 of Formula VIII may be replaced by OH or SH. This results in a reactive OH or SH group available for the attachment of functional and/or ancillary groups.

A further group of structural formulas for R^2 of Formulae II and III is that defined by Formula IX:

$$X'—R^8—Z' \qquad (IX)$$

In Formula IX, R^8 is a group having the formula

$$(CH_2)_o—R^9—(CH_2)_p \qquad (X)$$

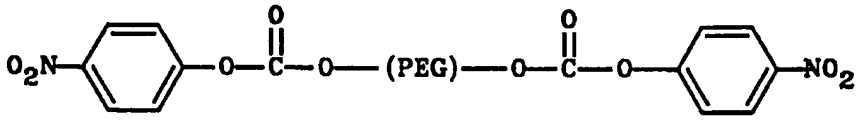
in which R^9 a cleavable group bearing the same definition as R^7 of Formulas IV and V above, *i.e.*, either a disulfide group S—S, or an ester group oriented in either direction, *i.e.*, C(O) —O or O—C(O). The indexes o and p are the same or different and are either zero or a positive integer, such that the sum of o + p is at least 2.

The symbols X' and Z' in Formula IX are the same or different and are inert linking groups of scope similar to the inert linking groups of the previous formulas. Preferred examples of X' and Z' are NH—C(O), C(O) —NH, NH—C(S) and C(S) —NH.

In accordance with these various formulas, the number and arrangement of functional and or ancillary groups on a single construct of either Formula II or Formula III may vary considerably. The number of functional groups will equal the product of m × n. The number of ancillary groups will equal the product of s × t. In general, constructs will be those in which either one or both products are greater than 10.

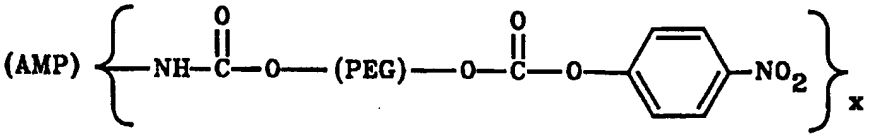
Constructs in accordance with the present invention may be synthesized in accordance with conventional linkage reactions that are well known among those skilled in the art. An example, in which the framework R^1 is functionalized with multiple amine groups, such as polylysine, is offered below. In this example, the backbone is referred to as (AMP)($—NH_2)_x$, "AMP" denoting the amplifying effect of permitting the attachment of a multitude of spacers and functional groups and/or ancillary groups, and x representing a number corresponding to n in Formulae II and III.

The attachment of a polyethylene glycol (PEG) spacer to the amplifier may be achieved by using an activated ester of PEG, such as an α,ω-bis-p-nitrophenoxy ester of PEG:



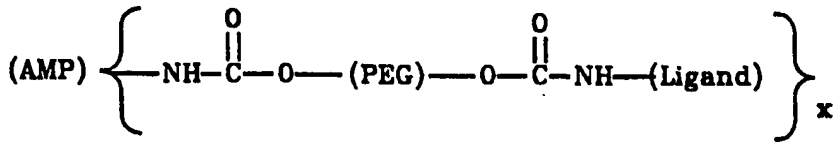
(XI)

in which "PEG" is polyethylene glycol minus the terminal hydroxyl groups, as defined above. An excess of this derivatized PEG ester X is reacted with (AMP)($—NH_2)_x$ to yield the intermediate:



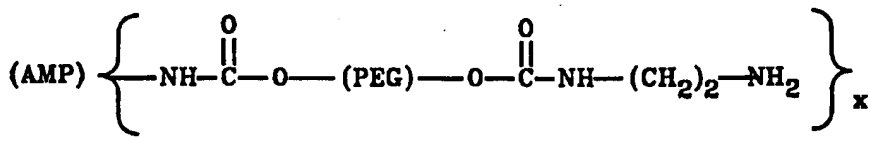
(XII)

The intermediate is then reacted with an excess of $H_2N—$ (Ligand), which denotes the ligand of the functional group derivatized to contain a reactive amine group. The product is the construct:



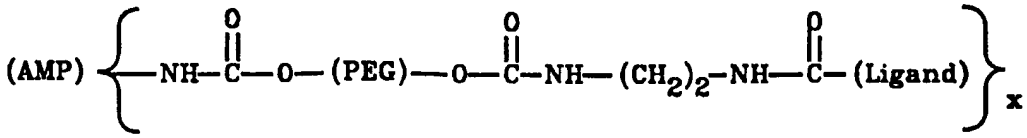
(XIII)

In an alternative scheme to a similar product, the above intermediate XI is converted to a second intermediate with a terminal amine group, by reaction with a diamine such as NH_2—$(CH_2)_2$—NH_2. The second intermediate has the structure:



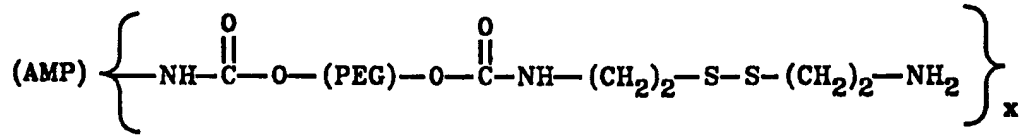
(XIV)

This intermediate XIV is then reacted with a carboxyl-activated ligand, such as, for example, an anhydride of the ligand, to produce a construct having the formula:



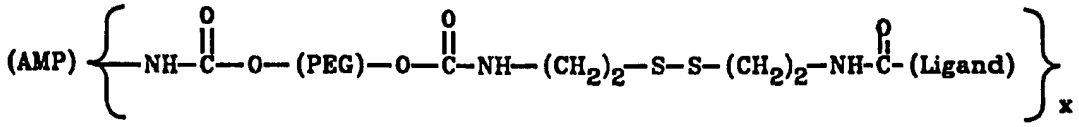
(XV)

A cleavable group such as disulfide can be introduced by reacting the intermediate X with a diamine containing an internal disulfide, such as cystenamine disulfide, NH_2—$(CH_2)_2$—S—S—$(CH_2)_2$—NH_2, to yield the further intermediate:



(XVI)

This may then be reacted with a carboxyl-activated ligand, to yield:



(XVII)

Many further alternatives to these schemes exist. To produce constructs containing cleavable esters in the spacers without PEG, for example, an amine- or hydroxyl-containing amplifying polymer can be derivatized to produce carboxylic acid groups as the functional groups. This is readily achieved by reacting the polymer with maleic, succinic or

glutaric anhydride using established procedures. A derivatized ligand to combine with the derivatized polymer can be formed by reacting a ligand bearing an isothiocyanate group with an amino alcohol, $HO(CH_2)_nNH_2$, to place a terminal hydroxyl group on the ligand. The carboxylic acid group on the derivatized polymer can then be activated by conventional methods using such agents as dicyclohexylcarbodiimide or carbonyldiimidazole, and reacted with the derivatized ligand to achieve the ester linkage. The section of the construct between the amplifying polymer and the ligand serves as the spacer, and the length of the spacer is determined by the number of CH_2 groups in the amino alcohol used to derivatize the ligand.

In an alternate scheme which produces a reverse ester, the ligand is derivatized with an aminocarboxylic acid, $HO_2C(CH_2)_2NH_2$, rather than an amino alcohol. The resulting carboxylic acid-derivatized ligand is then activated with dicyclohexyl-carbodiimide or carbonyldiimidazole and coupled directly to a hydroxyl-containing amplifying polymer.

In either of these two schemes, a selected fraction of the amine or hydroxyl groups that are native to the amplifying polymer can be protected if desired, to avoid interference with the coupling reactions. This is readily achieved by conventional methods, notably acetylation with acetic anhydride.

Ligands with functional groups for attachment to the spacer can be prepared by conventional methods. Well-known ligands for example are readily derivatized by methods known to those skilled in the art. It is preferable to select a ligand that retains all or most of its binding affinity even after derivatization.

Reaction Variations

As noted above, one approach to preparing the polyvalent presenters of the present invention involves the formation of combinatorial libraries, which consist of an array of synthetic polyvalent presenters, wherein the polyvalent presenters in the array differ from one another in terms of their compositions, structures, properties, functions, *etc.* In making the arrays of polyvalent presenters, one can vary, *inter alia*, the chemical structure of the framework component, the chemical structure of the functional group component, the chemical structure of the ancillary group, the chemical structure of the spacer group; the chemical nature of the framework component, the chemical nature of the functional group component, the chemical nature of the ancillary group, the chemical nature of the spacer

group; the amount of framework component delivered, the amount of functional group component delivered, the amount of ancillary groups delivered, the amount of spacer group delivered; the number and amount of different framework components delivered, the number and/or amount of different functional group components delivered, the number and/or amount of different ancillary groups delivered, the number and/or amount of different spacer groups delivered; the nature and number of the linkages between the various components (*e.g.*, the nature of the linkages of the spacer group); the reaction parameters (*e.g.*, reactant solvents, reaction temperatures, reaction times, reaction initiators, reaction catalysts, the atmospheres in which the reactions are carried out, the rates at which the reactions are quenched, *etc.*); the stoichiometry of the various components; the order in which the different components are delivered, *etc.* The various reactant components (*e.g.*, the framework component, the functional group component, the ancillary group, the spacer group, *etc.*) and the various methodologies which can be employed are described in greater detail hereinbelow.

In one approach, the activated polymer is reacted with one functional group in a manner that consumes substantially all of the activating groups on the polymeric framework. In this embodiment, the functional group to activating group stoichiometry is at least 1:1. An excess of functional group may be added if a 1:1 ratio is insufficient to substantially consume all of the activating groups.

In another approach, the functional group can be added in an amount insufficient to consume all of the activating groups. Following reaction with the first functional group, either a second functional group or an ancillary group can be delivered and reacted. Also encompassed within this embodiment is the addition of more than one functional group, either as a mixture or sequentially, followed by the addition of an ancillary group or, alternatively, more than one ancillary group as a mixture or sequentially.

In another approach, the activating groups are substantially consumed by the addition of a sufficient amount of a functional group. Following attachment of the first functional group to the framework component, the framework component is reactivated and a second functional group or an ancillary group is added. At either step of the reaction scheme of this embodiment, the functional group(s) may comprise an individual functional group, a mixture of different functional groups, or a mixture of functional groups and

ancillary groups, wherein the ancillary groups may comprise either a single ancillary group or a mixture of ancillary groups.

In yet another approach, the activating groups are substantially consumed by the addition of a mixture of at least two functional groups or a mixture of at least one functional group and at least one ancillary group.

In a still further approach, less than substantially all of the activating groups are reacted with a mixture of at least one functional group, at least one ancillary group or at least one functional group and at least one ancillary group. Following this first step, the remaining activating groups are substantially consumed by the addition of a further functional group, ancillary group or mixture of functional and ancillary groups.

In certain embodiments, the functional group(s), ancillary group(s) and/or mixtures of the functional and ancillary group(s) are added as dry powders or neat liquids to a solution of the activated framework. In other embodiments, solutions of the functional groups, ancillary groups and/or mixtures of functional and ancillary group(s) are added to a solution of the activated polymer. In still other embodiments, solutions of the functional group(s), ancillary group(s) or mixtures of the functional and ancillary group(s) are added to the polymers, wherein the polymer is present as a neat liquid or a dry powder.

Solvents of used in this aspect of the invention include any solvent is compatible with the presence of the activating groups (*i.e.*, does not react with the activated framework in a significant amount) and the nature of the reaction between the activated polymer and the functional and or ancillary groups. Such solvents include, for example, water, dimethylsulfoxide (DMSO), dimethylformamide (DMF), alcohols, ethers, ketones, hydrocarbons, aromatic hydrocarbons and mixtures, in any proportions, of these solvents.

In still other embodiments, the temperature can be controlled to control the rate of reaction between the activated framework and the functional group(s) and/or ancillary group(s). In other embodiments, other reaction parameters (*e.g.*, reactant solvents, reaction temperatures, reaction times, reaction initiators, reaction catalysts, the atmosphere in which the reactions are carried out, the pressure at which the reactions are carried out, the rates at which the reactions are quenched, *etc.*) can be varied and optimized.

In yet further embodiments, a "handle" on the functional group or the ancillary groups is activated by means of an activating group as described above and reacted with either an activated group on the framework or an unactivated moiety on the framework.

As used herein "handle" is used to refer to a reactive group such as, for example, carboxyl (acid or salt), hydroxyl, sulfhydryl, amide, carbamate, amine, ketone, aldehyde, olefin, diene, aromatic ring, *etc.* Appropriate combinations of framework-bound reactive moieties and functional group or ancillary group handles will be apparent to those of skill in the art.

Those of skill in the art will appreciate that although the foregoing discussion relates to the use of an activated polymer and a functional group, all of the foregoing embodiments are fully applicable to the methods involving the use of a monomer (*e.g.*, an underivatized monomer, a monomer derivatized with a spacer group, a monomer derivatized with an ancillary group either directly or indirectly through a spacer group, *etc.*) and a functional monomer (*e.g.*, a monomer derivatized with a functional group, *i.e.*, R^3, either directly or indirectly through a spacer group).

Substrate and Delivery Systems

"Substrate" as used herein refers a material having a rigid or semi-rigid surface or, alternatively, a material having dimples, wells, containers, trenches, *etc.* In many embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments, it may be desirable to physically separate synthesis regions for different materials with, for example, dimples, wells, raised regions, etched trenches, or the like. In some embodiments, the substrate itself contains wells, raised regions, etched trenches, *etc.*, which form all or part of the synthesis regions.

More particularly, the substrate can be organic, inorganic, biological, nonbiological or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, *etc.* The substrate can have any convenient shape, such a disc, square, sphere, circle, *etc.* The substrate is preferably flat, but may take on a variety of alternative surface configurations. For example, the substrate may contain raised or depressed regions on which the synthesis of diverse polyvalent presenters takes place. The substrate and its surface preferably form a rigid support on which to carry out the reactions described herein. The substrate may be any of a wide variety of materials including, for example, polymers, plastics, pyrex, quartz, resins, silicon, silica or silica-based materials, carbon, metals, inorganic glasses, inorganic crystals, membranes, *etc.* Other substrate materials will be readily apparent to those of skill in the art upon review of this disclosure. Surfaces on the solid substrate can be composed of

the same materials as the substrate or, alternatively, they can be different, *i.e.*, the substrates can be coated with a different material. Moreover, the substrate surface can contain thereon an adsorbent (for example, cellulose) to which the components of interest are delivered. The most appropriate substrate and substrate-surface materials will depend on the class of materials to be synthesized and the selection in any given case will be readily apparent to those of skill in the art. In a preferred embodiment, suitable substrates include, for example, microtiter plates (*e.g.*, having 96 wells) or a test tube holder containing therein test tubes in an amount sufficient to hold each of the polyvalent presenters of the array.

The reactant components in the individual reaction regions must often be prevented from moving to adjacent reaction regions. Most simply, this can be ensured by leaving a sufficient amount of space between the reaction regions on the substrate so that the various components cannot interdiffuse between reaction regions. Moreover, this can be ensured by providing an appropriate barrier between the various reaction regions on the substrate. In one approach, a mechanical device or physical structure defines the various reaction regions on the substrate. A wall or other physical barrier, for example, can be used to prevent the reactant components in the individual reaction regions from moving to adjacent reaction regions. This wall or physical barrier can be removed after the synthesis is carried out. One of skill in the art will appreciate that, at times, it may be beneficial to remove the wall or physical barrier before screening the array of materials.

In another approach, a hydrophobic material, for example, can be used to coat the region surrounding the individual reaction regions. Such materials prevent aqueous (and certain other polar) solutions from moving to adjacent reaction regions on the substrate. Of course, when non-aqueous or nonpolar solvents are employed, different surface coatings will be required. Moreover, by choosing appropriate materials (*e.g.*, substrate material, hydrophobic coatings, reactant solvents, *etc.*), one can control the contact angle of the droplet with respect to the substrate surface. Large contact angles are desired because the area surrounding the reaction region remains unwetted by the solution within the reaction region.

In the delivery systems of the present invention, a small, precisely metered amount of each reactant component is delivered into each reaction region. This may be accomplished using a variety of delivery techniques. For instance, the various reactant components can be delivered to the reaction regions of interest from a dispenser in the form

of droplets or powder. Conventional micropipetting apparatuses can, for example, be adapted to dispense various droplet volumes from a capillary. The dispenser can also be of the type employed in conventional ink-jet printers. Such ink-jet dispenser systems include, for example, the pulse pressure type dispenser system, the bubble jet type dispenser system and the slit jet type dispenser system. These ink-jet dispenser systems are able to deliver various droplet volumes. Moreover, such dispenser systems can be manual or, alternatively, they can be automated or semi-automated using, for example, robotics techniques.

In other embodiments, the reactant solutions can be delivered from a reservoir to the substrate by an electrophoretic pump. In such a device, a thin capillary connects a reservoir of the reactant with the nozzle of the dispenser. At both ends of the capillary, electrodes are present to provide a potential difference. As is known in the art, the speed at which a chemical species travels in a potential gradient of an electrophoretic medium is governed by a variety of physical properties, including the charge density, size, and shape of the species being transported, as well as the physical and chemical properties of the transport medium itself. Under the proper conditions of potential gradient, capillary dimensions, and transport medium rheology, a hydrodynamic flow will be set up within the capillary. Thus, bulk fluid containing the reactant of interest can be pumped from a reservoir to the substrate. By adjusting the appropriate position of the substrate with respect to the electrophoretic pump nozzle, the reactant solution can be precisely delivered to predefined reaction regions on the substrate.

The dispenser can be aligned with respect to the appropriate reaction regions by a variety of conventional systems. Such systems, which are widely used in the microelectronic device fabrication and testing arts, can deliver droplets of reactant components to individual reaction regions at rates of up to 5,000 drops per second. The translational (X-Y) accuracy of such systems is well within 1 μm. The position of the dispenser stage of such systems can be calibrated with respect to the position of the substrate by a variety of methods known in the art. For example, with only one or two reference points on the substrate surface, a "dead reckoning" method can be provided to locate each reaction region on the substrate. The reference marks in any such systems can be accurately identified by using capacitive, resistive or optical sensors. Alternatively, a "vision" system employing a camera can be employed.

In another embodiment, the dispenser can be aligned with respect to the reaction region of interest by a system analogous to that employed in magnetic and optical storage media fields. For example, the reaction region in which the reactant component is to be deposited is identified by its track and sector location on the disk substrate. The dispenser is then moved to the appropriate track while the disk substrate rotates. When the appropriate reaction region is positioned below the dispenser, a droplet of reactant solution is released.

In some embodiments, the reaction regions may be further defined by dimples in the substrate surface. This will be especially advantageous when a head or other sensing device must contact or glide along the substrate surface. The dimples can also act as identification marks directing the dispenser to the reaction region of interest.

Those of skill in the art will readily appreciate that the structure, *i.e.*, composition, of polyvalent presenters can be determined from the synthesis history or can be obtained by sequencing, mass spectrometry, deconvolution, encoding, *etc.* Such evaluation strategies are described, for example, by Thompson *et al.*, *Chem. Rev.*, 1996, *96*, 555-600, the teachings of which are incorporated herein by reference.

PHARMACEUTICAL COMPOSITIONS

The present invention also pertains to pharmaceutical compositions for polyvalently presenting an agent for therapy. The pharmaceutical compositions contain a polyvalent presenter, as described in detail above, and a pharmaceutically acceptable carrier. In one embodiment, the polyvalent presenter can have a formula as follows:

$$(Y)\text{-}(X\text{-}A)_n$$

wherein Y is a framework, X is a direct bond or a linker, A is a presented functional group, and n is greater than ten and is an integer selected such that the presented groups can interact with a collection of target binding sites B. The presenter itself can serve as its own pharmaceutically acceptable carrier. In one embodiment, the polyvalent presenter is made, e.g., n is selected and the - (X-A) moieties are attached to Y, such that the polyvalent presenter conforms to an interface containing a collection of target binding sites B and blankets the collection of target binding sites B upon administration to a subject. In another embodiment, X is a linker group which is an independent moiety and is not part of Y or A and n is greater than ten and is an integer selected such that the polyvalent presenter conforms to a collection of target binding sites B upon administration to a subject. In yet another embodiment, Y is a polymeric framework, A is a presented functional group and X is

a linker group and n is greater than ten and is an integer selected such that the polyvalent presenter conforms to a collection of target binding sites B upon administration to a subject.

The language "pharmaceutically acceptable carrier" is intended to include substances capable of being coadministered with the polyvalent presenter(s) and allows it to perform their intended function. Examples of such carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. The use of such media for pharmaceutically active substances is well known in the art. Any other conventional carrier suitable for use with the polyvalent presenter(s) also falls within the scope of the present invention. It should be understood that many of the polyvalent presenters of the present invention are water soluble. Accordingly, formulation of the polyvalent presenters of the invention into pharmaceutical compositions is rendered more facile with fewer problems than with other materials that are less water soluble.

The language "therapeutically effective amount" of the polyvalent presenter is that amount necessary or sufficient to perform its intended function within the subject. The therapeutic effective amount can vary depending on such factors as the type of site being targeted, the type of components (e.g., frameworks, e.g., linkers, e.g., groups A) employed, the size of the subject, or the severity of the symptom(s). One of ordinary skill in the art would be able to study the aforementioned factors and make the determination regarding the effective amount of the polyvalent presenter without undue experimentation. An *in vitro* or *in vivo* assay also can be used to determine an "effective amount" of the polyvalent presenter. The ordinarily skilled artisan would select an appropriate amount of the polyvalent presenter for use in the aforementioned assay.

The data obtained from cell culture assays and animal studies can be used in formulating an appropriate range of dosages for use in subjects. The dosage of such agents lies preferably within a range of circulating or tissue concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a concentration range that includes the IC50 (i.e., the concentration of the test modulating agent which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The regimen of administration also can affect what constitutes an effective amount. The polyvalent presenter can be administered alone or in conjunction with other agent(s). Further, several divided dosages, as well as staggered dosages, can be administered daily or sequentially, or the dose can be continuously infused. Further, the dosages of the

polyvalent presenter(s) can be proportionally increased or decreased as indicated by the exigencies of the therapeutic situation.

Pharmaceutical compositions for use in accordance with the present invention may also be formulated in conventional manner using one or more physiologically acceptable carriers or excipients as the pharmaceutically acceptable carrier. Thus, the polyvalent presenters and their physiologically acceptable salts and solvates may be formulated for administration by, for example, topical application, injection, inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

As such, the agents of the invention can be formulated in a manner appropriate for a specific mode of administration chosen, including, e.g., systemic and topical or localized administration. Techniques and formulations generally may be found in Remmington's Pharmaceutical Sciences, Meade Publishing Co., Easton, PA. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the polyvalent agents of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the agents may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

For oral administration, the pharmaceutical compositions of polyvalent presenters may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid compositions for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid compositions may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The compositions may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Compositions for oral administration may be suitably formulated to give controlled release of the active modulating agent.

For administration by inhalation, the compositions for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g.,

dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the polyvalent agent and a suitable powder base such as lactose or starch.

The agents can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The agents may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described above, the agents may also be formulated as a depot composition. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the modulating agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through nasal sprays or using suppositories. For topical administration, the oligomers of the invention are formulated into ointments, salves, gels, or creams as generally known in the art.

In embodiments wherein the polyvalent presenter is large and does not pass passively and rapidly through hydrophobic membranes, injection or inhalation may be more appropriate than ingestion or transdermal delivery. These issues of delivery are related to those for protein-based drugs. In embodiments where the polyvalent presenter passes through the GI tract, appropriate delivery methods known in the art may be employed.

The compositions may, if desired, be provided in a pack or dispenser device, or as a kit with instructions. The composition may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil,

such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration, e.g., for use in the methods described herein.

COLLECTION OF POLYVALENT PRESENTERS

The present invention also pertains to collections of polyvalent presenters. These collections can be used in the methods of the invention. A collection of polyvalent presenters includes more than one polyvalent presenter combined together. The collections can have as many different polyvalent presenters that allow it to perform its intended function.

The present invention even further pertains to formulations, e.g., therapeutic compositions containing a collection of polyvalent presenters and a pharmaceutical carrier as defined above. The formulation containing a collection of polyvalent presenters can be designed such that different polyvalent presenters are selected for their ability to impart a particular characteristic into the overall formulation.

The collection of polyvalent presenters can be in the form of a polydispersion. The discussion below will use the term "polydispersion" for the collection but it should be understood that it also pertains broadly to other forms of collections of polyvalent presenters of the present invention.

A polydispersion of polyvalent presenters contains more than one different polyvalent presenter. The polydispersion of different polyvalent presenters can be a collection of sizes because of such factors as differences in the length of the frameworks and degree of substitution with groups A, and/or difference in group A/linker combinations. There also may be differences in the precise positions of group A attachments. Each member of the collection can impart different properties to an overall formulation, which can be used as an advantage in treating a disease or condition. For example, lower-molecular-weight members of the collection may access their targets of action more rapidly than do higher molecular weight members. At the same time, high-molecular-weight members may have a longer duration of action than lower-molecular-weight members due to longer residence time at the site of action and/or lifetime in the relevant compartment. These differences can be used to an advantage in treating a disease or condition in that polyvalent presenters can have as a collection, properties that are overall superior to those of a corresponding number of identical species, e.g., a combination of both rapid onset of action and prolonged duration of action, or different.

METHODS FOR TREATING A DISEASE OR CONDITION

The present invention also pertains to a method(s) for treating a disease or condition. The method involves administering to a subject a plurality of Groups A such that the treatment of the disease or condition occurs. In one embodiment, the treatment of the

disease or condition can occur by the conformal interface interaction of a polyvalent presenter with a collection of binding sites B or the conformal interface interactions of a plurality of polyvalent presenters with collections of binding sites B within the subject. The conformational surface interaction(s) result in the blanketing of either a collection of binding sites B or an array of target binding sites B within the subject. In another embodiment, the polyvalent presenter that facilitates treatment meets a set of criteria. The criteria are as follows:

the groups A are functional and act as a drug, alone, or in combination with the framework such as, e.g., a compound that may be of insufficient affinity alone but amplified as a result of the present invention;

the presentation of the groups A attached to the framework provide an additional benefit to the interaction relative to the presentation of a single group A to a plurality of binding sites B; and

the additional benefit is a synergistic benefit in that the benefit is greater than the additive benefit that would have been provided by a collection of monomers of the same group A dispersed in a homogenous solution.

Examples of additional benefits include a benefit selected from the group consisting of the provision of a sufficient biological effect at a lower concentration of groups A, the enhancement of specificity for a targeted versus non-targeted site, and the enhancement of biological potency. In a preferred embodiment, the polyvalent presenter provides at least two additional benefits and in an even more preferred embodiment at least three additional benefits. The benefits can be selected from the above list and also from those benefits described below in the sections describing the pharmacodynamics and/or the mechanisms related to the functioning of the polyvalent presenters, e.g. positive cooperativity, entropic enhancement of binding and provision of steric inhibition.

For the methods for treating a disease or condition, the binding sites B are located such that a polyvalent interaction can occur. For example, the binding sites B with which the groups A of the polyvalent presenter interact can be intracellular, or extracellular (e.g., on the cell surface or on extracellular matrix) or localized within cellular membranes. The polyvalent presenters of the present invention can have a variety of functions in terms of facilitation of treatment, they can be used to modulate (e.g., up or downregulate) a state or condition associated with polyvalency, for example, an unwanted state (e.g., fertilization) or a disease state (e.g. infection). The subject presenters can be used to target specific biological events of the disease or condition (e.g., prophylactic prevention of cell-pathogen attachment) or can be used to treat specific disease states (e.g., a patient presenting with a pathogenic infection to control or reverse the spread of infection). Likewise, the subject presenters have a variety of *ex vivo* applications in the modulation of such states.

Modulation of specific biological events

In certain embodiments, the polyvalent presenters of the present invention can be used, for example, to modulate cell-cell interactions. Numerous biological processes require cell-cell interactions and the subject presenters can either promote or inhibit such interactions. One particular instance involves the binding of cells to other cells such as, for example, neutrophils and arterial endothelial cells. The attachment of a neutrophil initially suspended in rapidly flowing blood to the endothelial cells closest to a site of inflammation occurs through polyvalent interactions. (A. Varki, *J. Clin. Invest.* **1997**, *99*, 158-162; J. B. Lowe, P. A. Ward, *J. Clin. Invest.* **1997**, *99*, 822-826; M. A. Gimbrone, T. Nagel, J. N. Topper, *J. Clin. Invest.* **1997**, *99*, 2062-2070.) Signaled by nearby inflammation, this initially rapidly transported neutrophil adheres to the surface of the endothelial cells, and then rolls slowly (10 - 20 μm/min); endothelial cells are those that line the interior of the blood vessels. This rolling is mediated by interactions between multiple E- and P-selectins on the surface of the endothelial cell (these selectins are not normally present on the surface of these cells; they are induced by cytokines during inflammation) and multiple glycoproteins displaying sialyl Lewis X (a tetrasaccharide) on the surface of the neutrophil. (M. A. Gimbrone, T. Nagel, J. N. Topper, *J. Clin. Invest.* **1997**, *99*, 2062-2070) In addition, L-selectin, present on the surface of the neutrophil, interacts with sialyl Lewis X, present on the endothelial cell. The valency of this set of interactions may significantly influence the kinetics, dynamics and specificity of neutrophil recruitment. (L. L. Kiessling, N. L. Pohl, *Chem. & Biol.* **1996**, *3*, 71-77). Table 2 provides other examples of cell-cell interactions.

Table 2. Examples of Polyvalent Cell-Cell Interactions

Cell-1	Molecule on Cell-1	Cell-2	Molecule on Cell-2
Neutrophil	L-selectin, P-selectin	Endothelial Cell	sulfated sialyl Le^x
Neutrophil	Sulfated sialyl Le^x	Endothelial Cell	E-Selectin
	Neutrophil Cell-Adhesion Molecule (NCAM) such as L1, NCAM-H, CD24 and α_6-integrin		
Neutrophil	E-selectin Cadherins	Neutrophil	Sulfated sialyl Le^x
T-cell	T-cell Receptor	Antigen Presenting Cells	Major Histocompatibility Complex (MHC)
	CD3		MHC
	CD28		
Platelet	Ia/IIa	Endothelial Cell	Collagen
Platelet	IIb/IIIa	Platelet	IIb/IIIa
Tumor Cell	GalTase	Endothelial Cell	GlcNAc
Sperm	GalTase	Egg	GlcNAc
Aging Red Blood Cell	Desialylated glycoproteins	Hepatocyte	C-type lectin

In another embodiment the subject polyvalent presenters can be used to inhibit another type of cell-cell interaction, e.g., attachment. For example the attachment of leukocytes to endothelial cells is mediated by a family of adhesion molecules known as selectins. The selectins are transmembrane glycoproteins expressed on platelets (P-selectin), leukocytes (including neutrophils and other lymphocytes, L-selectin) and on endothelial cells (E- and P-selectins). The selectins mediate rolling interactions on endothelial vessel walls, an interaction that ultimately leads to leukocyte extravasation (Kretzschmar, *et al.*, 1995. Tetrahedron 51:13015). The subject presenters will be useful in inhibiting both initial attachment events and events which follow thereafter, e.g., leukocyte migration. The polyvalent presenters of the present invention can be used to interfere with neutrophil-endothelial cell interaction and, thus, modulate states associated with neutrophil adhesion, such as, e.g., inflammation, adult respiratory distress syndrome, rheumatoid arthritis, septic shock, and reperfusion injury.

In another embodiment the subject polyvalent presenters can be used to inhibit platelet-platelet interaction. Platelet aggregation plays a salient role in thrombotic or thromboembolic events. ADP, thrombin, epinephrine, or collagen agonists expose fibrinogen-binding sites on the plate protein, GPIIb-IIIa. Small peptides, such as RGD or HHLGGAKQAGDV (H_{12}) contain fibrinogen recognition motifs and can block fibrinogen binding to these sites. Peptide binding to GPIIb-IIIa induces the expression of cryptic epitopes on the GPIIb-IIIa complex (Gawaz, *et al.*, 1996. Arterioscler Thromb Vasc. Biol.

16:621). Platelet granules are then released; one of the major glycoproteins of granules is thrombospondin, which has been shown to mediate platelet aggregation. (Gawaz, *supra*). Groups A can be chosen to inhibit any of the interactions leading to platelet aggregation. In a preferred embodiment, the interaction of GPIIb-IIIa and a fibrinogen recognition motif is modulated. In certain embodiments it will be desirable to induce hypocoagulation in a subject, while in other embodiments it will be desirable to induce a hypercoagulative state in a subject.

In another embodiment the polyvalent presenters of the instant invention can be used to reduce the ability of cancer cells to metastasize. Metastasis involves the detachment of tumor cells from primary sites, their invasion into neighboring tissues, and their settlement at secondary sites, and the subject polyvalent presenters can act at any of these stages. The tropism of cancer cells for various organs can be mediated by lectins of the invaded organs or of the tumor cells (Matrosovich. 989. FEB 252:1,2:1-4; Beuth, *et al.*, 1988. Clin. Exp. Metastasis. 6:115-120). For example, β-lactosyl clusters have been described as potential tumor metastasis inhibitors (Dean, *et al.*, 1993. Carbohydrate Research 245:175). Accordingly, polyvalent presenters can be made which incorporate modulators of these recognition events as groups A.

In another embodiment the subject presenters can be used to modulate infection. The initial step in most cell-pathogen interactions involves attachment. This is true for all viruses. One example of adhesion of a virus to the surface of a cell is influenza and bronchial epithelial cells. As the first step in infection, influenza virus attaches to the surface of a bronchial epithelial cell. The attachment occurs by interaction between multiple trimers of the hemagglutinin (HA, a lectin found densely packed on the surface of the virus, about 2-4 copies/100 nm^2 or 600-1200 per virus particle) and multiple moieties of sialic acid (SA, the terminal sugar on many glycoproteins and one that is also arranged densely on the surface of the target cell, ~ 50-200 copies/100 nm^2). Table 3 provides other examples of cell-virus interactions (wherein **P** = protein; S = sugar (glycoprotein or glycolipid). A growing number of viruses are found to use more than one type of interaction for attachment to target cell.

Table 3. Examples of Ligands and Receptors on Surfaces of Virus and Host Cell

DNA Viruses	Disease	Molecule on Virus	Molecule on Host
Papovaviridae *Polyomavirus* Polyomavirus	DNA-containing tumor virus	VP1	S: NeuAc(α2,3)Gal(β1,3)GalNAc S: sialyloligosaccharides
Adenovirus *Mastadenovirus* Human adenovirus	Acute respiratory disease, pneumonia, gastroenteritis (Also a useful vector for gene therapy)	P: Residues 1-141 of E3gp (fiber knob protein) P: RGD base of the Penton base protein (different from fiber protein)	P: Class I MHC P: Penton base interacts with β_2 integrins
Herpesviridae-alpha *Herpes Simplex Virus* type 1	Herpes Simplex	P: glycoprotein E,D	P: gD, gH (proposed 2 step attachment)
Varicella-Zoster Virus	Chicken Pox Shingles	P: Glycoprotein I (gE) (gE dimer resembles Fc receptor)	
Herpesviridae-beta *Cytomegalovirus* Human cytomegalovirus	Major cause of infant blindness and retardation; implicated in AIDS and cancer (Kaposi sarcoma)	P: gC-11, 86 kDa gp residues 204-297 of H301 α-domain gene resembling Class I MHC α3	S: Heparan sulfate P: Class I HLA MHC through β_2-microglobulin
Lymphocryptovirus Epstein-Barr virus	Mononucleosis	P: gp 350 P: gp3	P: C3d receptor CR2 (CD21) of B lymphocyte - resembling C3 complement fragment C3d
Poxviridae *Orthopoxvirus* Vaccina virus	Used for small pox vaccination; very similar antigenic patterns compared with variola virus	P: Residues 71-80 of VGF protein - resembling EGF α	P: Epidermal growth factor receptor. Some evidence to the contrary, however.
Parvoviridae *Parvovirus* Canine Parvovirus	This virus is a model for the human strain, which causes erythema infectiosum ("fifth disease"); the human form may cause hydrops fetalis or fetal death if contracted during pregnancy	P: GP1-anchored protein	P: 3201 T-cells P: surface of precursors of erythrocytes in the bone marrow (infection can cause anemia)

Hepadnavirus *Hepatitis type B* Human Hepatitis B Virus	Serum hepatitis, a chronic infection that results in liver failure and death in 1-2% of cases	P: PreS portion of Env protein P: Residues 21-47 of preS portion of Env protein P: PreS1 sequence of large S protein	P: IL-6 receptor, pre-S1 binding protein P: Hepatocyte receptor for polymerized serum albumin P: Hepatocyte receptor for polymeric IgA P: Sialoglycoprotein P: Mediated attachment through soluble IL6, gaining entry into non-liver cells indirectly S: Asialoglycoprotein receptor
Avian hepatitis B virus	Serum hepatitis in birds		P: glycoprotein 180
RNA viruses			
Picornaviridae *Enterovirus* Poliovirus	Polio	P: Residues 95-105 of VP1 capsid protein	P: Member of immunoglobulin superfamily, not CD44 as previously reported.
Rhinovirus Human rhinovirus	Common Cold	P: Residues of VP1 and VP3 major capsid proteins lining a canyon on the virus surface	P: ICAM-1
Cardiovirus Encephalomyocarditis virus (Mengo fever)	Fevers, rarely fatal; pathogenic for many animals (has caused outbreak of fatal myocarditis in pigs, for instance)	P: Residues of VP1 and VP3 major capsid	P: Sialoglycoprotein P: VCAM-1
Aphthovirus Foot and Mouth disease Virus	A highly infectious disease of cattle, sheep, pigs and goats (10-70% mortality); usually non-fatal in humans, but readily contracted from animals (causes high fevers)	P: NS28 glycoprotein P: Residues 145-147 (RGD) and 203-213 of VP1 protein P: Residues 133-158 and C-terminal region of VP1 protein	P: Integrins
Reoviridae *Rotavirus* Human Rotavirus	Single most important cause of gastroenteritis and diarrhea of human infants worldwide: 500 million+ cases annually, resulting in 5 million deaths	P: C-terminal portion of hemagglutinin	1. P: K562 erythroleukemia cells? 2. P: β adrenergic receptor? 3. P: Sialoglycoproteins in the vili of cells in the small intestine 4. S: Sialic acids
Togaviridae *Alphavirus* Semliki Forest virus	Fever, encephalitis	P: Nucleocapsid	P: Class I HLA and H-2 MHC molecules

Lactate dehydrogenase-Elevating virus	Fever, encephalitis	P: Envelope glycoprotein (VP-3P)	P: Class II 1a MHC molecule of macrophage
Sindbis	Related to the western equine encephalitis virus, which has ~5% mortality	P: E1-E2	P: High affinity laminin receptor
Rubivirus Rubella	Rubella (German Measles)		S: Unidentified glycolipid[57, 58]
Orthomyxoviridae *Influenzavirus* Influenza virus	Major cause of various forms of human respiratory disease, including fatal pneumonia	P: Hemagglutinin Protein (HN or H protein)	P: HN protein, liver cell S: Sialyloligosaccharides Influenza A S: NeuAc(α2,6)Gal(β1,4)GlcNAc S: NeuAc(α2,3)Gal(β1,3)GalNAc S: NeuAc(α2,3)Gal(β1,4)GlcNAc Influenza C S: 9-O-AcNeuAc
Paramyxoviridae *Paramyxovirus* Sendai virus	As a genus, Paramyxovirus is second only to respiratory syncitial virus in causing lower respiratory tract infections in young children	P: Hemagglutinin Protein	S: Sialyloligosaccharides S: NeuAc(α2,3)Gal(β1,3)GalNAc S: NeuAc(α2,8)NeuAc(α2,3)Gal(β1,3)GalNAc S: Ganglioside GD1a
Newcastle disease virus	Lower respiratory tract infections	P: Hemagglutinin-neuraminidase	S: Sialyloligosaccharides S: NeuAc(α2,3)Gal(β1,3)GalNAc
Morbillivirus Measles virus	Measles	P: Measles virus hemagglutinin	P: CD46
Pneumovirus Respiratory syncytial Virus	Leading cause of lower respiratory tract infections (bronchiolitis and pneumonia), possible role in sudden infant death syndrome, major cause of middle ear infections	P: Fusion protein; nucleocapsid	
Coronavirus OC43	Common cold Pharyngitis		S: $Neu5,9Ac_2$

Rhabdoviridae *Vesiculovirus* Vesicular stomatitis Virus		P: Glycoprotein	S: Phosphatidylserine S: Phosphatidyl inositol S: GM3 ganglioside
Lyssavirus Rabies virus	Rabies	P: Residues 151-238 of G protein – resembling Curare-mimetic neurotoxins	P: BHK-21 cells P: Acetylcholine receptor (residues 173-204 of α subunit) S: sialylated gangliosides
Retroviridae *Oncovirus C - Human* Human T-cell leukemia virus (HTLV-1)	Cancer – leukemia	P: Residues 246-253 of envelope glycoprotein – resembling both extracellular portion of HLA-B MHC molecule and IL2	P: Class I HLA MHC molecule P: Interleukin-2 receptor
Radiation leukemia Virus	Cancer – leukemia		P: T-cell receptor:L3T4 molecule complex
Amphotrophic retrovirus	Cancer – leukemia		P: CD34 on marrow progenitor cells
Oncovirus C - Avian Avian leukosis virus	Cancer		P: Low density lipoprotein receptor
Oncovirus C – Mammalian Murine leukemia virus (Moloney)	Cancer – leukemia	P: Envelope gp71	P: Lymphoma cell surface IgM P: 622 amino-acid hydrophobic protein of unknown function
Ecotropic murine Retrovirus	Cancer	P: Envelope glycoprotein	P: Basic amino acid transporter
Nonecotropic murine Leukemia virus	Leukemia	P: gp70 (surface)	

HIV-1	AIDS	P: gp120-resembling Ig heavy chain regions P: gp120-resembling Neuroleukin P: gp120-resembling vasoactive intestinal peptide	P: CD4 of T cell P: CD4 molecule interacting with class II HLA-DR MHC molecule S: Galactosyl ceramide (or closely related molecule on human colon epithelial HT29 cells) P: CR2, especially in EBV infected cells P: Chemokine receptors CXCR-4 (T-cell trophic) and CCR-5 (Macrophage trophic) (both are 7-transmembrane G-protein coupled receptors)
HIV-2	AIDS		P: CD4
Simian Immunodeficiency virus	Immunodeficiency syndrome analogous to AIDS in simians	P: SIV mac239	P: CD4
African swine virus		P: p12, p72	

Viral attachment to its host is the first step to viral infection, and involves the simultaneous association of multiple molecules on the surface of the virus with multiple molecules on the surface of its host cell (M. Tardieu, R. L. Epstein, H. L. Weiner, *Int. Rev. Cytol.* **1982**, *80*, 27-61). The molecules involved on the surfaces of the virus and cell that mediate attachment are most directly identified when this attachment is inhibited by either a high concentration of corresponding free monomer of that molecule or by monoclonal antibodies against that molecule. Such a strategy is used often when the ligand is a sugar or small molecule. A less direct characterization of the molecules involved in attachment comes from correlations of mutations (either man-made or natural) with an inability to bind successfully to the target cell; that is, if a mutation of a particular protein eliminates the ability of the virus to attach, it is involved in the attachment either directly or indirectly. This second strategy is often used for receptors or ligands that are proteins. Although a growing number of molecules on surfaces have been implicated in virus-cell recognition, there are often ambiguities. Because viral particles adhere to many substances non-specifically, it is often difficult to distinguish non-biospecific from biospecific binding. Some viral particles may enter a host cell by non-specific transport (the same mechanism that a cell uses to internalize small molecules). Some proteins on the surface of a virus can recognize more than one type of ligand on the cellular surface. Finally, certain viral particles may associate with a protein freely diffusing in solution, which in turn recognizes molecules on the surface of a cell (mediated attachment). Viruses bind to almost all classes of molecules on cellular surfaces: sugars (A. Varki, *Glycobiol.* **1993**, *3*, 97-130) (for example, polyoma (H. Fried, L. D. Cahan, J. C. Paulson, *Virol.* **1981**, *109*, 188-192) and orthomyxoviruses (J. C. Paulson, J. E. Sadler, R. L. Hill, *J. Biol. Chem.* **1979**, *254*, 2120-2124) recognize sialyloligosaccharides); phosphatidyl lipids (for example, vesicular stomatitis virus, VSV, recognizes phosphatidylserine and phosphatidylinositol (P. Mastromarino, C. Conti, P. Goldoni, B. Hauttecoeur, N. Orsi, *J. Gen. Virol.* **1987**, *68*, 2359-2369)); and proteins (for example, HIV recognizes CD4 (A. G. Dalgleish, P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, R. A. Weiss, *Nature* **1984**, *312*, 763-767), human rhinovirus recognizes intercellular adhesion molecule-1, ICAM-1 (J. M. Greve, G. Davis, A. M. Meyer, C. P. Forte, S. C. Yost, C. W. Marlor, M. E. Kamarck, A. McClelland, *Cell* **1989**, *56*, 839-847)).

The system for viral attachment and inhibition that has been most extensively studied in the context of polyvalency is the orthomyxovirus A (X-31) (an engineered strain of influenza) attaching to the surface of erythrocytes (W. J. Lees, A. Spaltenstein, W. J. E. Kingery, G. M. Whitesides, *J. Med. Chem.* **1994**, *37*, 3419-3433; M. Mammen, G. Dahmann, G. M. Whitesides, *J. Med. Chem.* **1995**, *38*, 4179-4190). This attachment occurs through multiple simultaneous interactions between viral HA and cellular SA. Accurate measurements of the affinity between influenza virus and erythrocyte have so far not been performed.

Another extensively studied virus-cell interaction is that between the protein gp120 on the human immunodeficiency virus (HIV) and the 60K glycoprotein CD4 on the surface of the T-cell. Large concentrations of solubilized CD4 (CD4 that has been chemically cleaved from the surface of a cell) inhibits attachment. HIV is an example of a virus that can use alternate molecules on the surface of cells: HIV can infect glioma and rhabdosarcoma cells, both of which lack CD4. Solubilized CD4 does not block attachment and subsequent infection of these cell types (P. R. Clapham, J. N. Weber, D. Whitby, K. McIntosh, A. G. Dalgleish, P. J. Maddon, K. C. Deen, R. W. Sweet, R. A. Weiss, *Nature* **1989**, *305*, 60-62).

There are many examples of mediated binding of virus to cells. One example involves Hepatitis B virus, that in some cases bind to aggregates of serum albumin; these aggregates in turn bind to receptors for albumin on the surface of hepatocytes (P. Pontisso, M. A. Petit, M. J. Bankowski, M. E. Peeples, *J. Virol.* **1989**, *63*, 1981-1988). A second well-studied example begins with the bivalent attachment of anti-virus antibodies to the surface of the virus (examples include Dengue virus, West Nile virus, and HIV) (R. M. H. V. Van, G. Hardie, *Immunochem.* **1976**, *13*, 503-507). Fc-receptors on the surface of the target cell then interact polyvalently with multiple Fc tails. This mechanism of attachment is the same as that used by macrophages in recognition of foreign pathogens: interestingly, where the former leads to infection, the latter leads to clearance.

Bacterial pathogens can be divided into two classes: intracellular (those that enter a cell and multiply there) and extracellular (those that live among cells, but not inside them). The mechanism of infection by those that are intracellular is similar to that of viruses: infection is initiated by attachment of the pathogen to the host cell (Tables 4-5). Bacteria that are extracellular often migrate to and collect in particular tissues. Concentration of

bacteria in specific tissues (tissue tropism) can occur by specific interactions between molecules on the surface of the bacteria and either molecules on the surfaces of cells in the preferred tissue, or components of the extracellular matrix characteristic of that tissue. Many specific interactions between bacteria and either host cell or extracellular matrix have been reviewed previously (I. Ofek, N. Sharon, *Curr. Topics Microbiol. Immunol.* **1990**, *151*, 90-113; I. Ofek, N. Sharon, *Infect. Immun* **1988**, *56*, 539-547). As with viruses, the specificity of the interaction is usually defined by the molecule that is the best inhibitor of an adhesion or agglutination assay. These assays are most often used when the interaction is between a lectin on the bacterial surface and a sugar on the either the host cell or the extracellular matrix (Table 4). At present, bacterial interactions appear more likely than viral ones to involve proteins as both ligand and receptor (Table 5). These protein-protein interactions are more difficult to study, mainly because the "monomer" is either not known, not available for study, or loses its "shape" when removed from the context of the membrane. As a result, most of the detailed studies of bacterial and viral interactions to date involve lectin-sugar interactions, and not protein-protein ones.

Enteric bacteria are those that take up residence in the gastrointestinal tract of mammals. In most cases, the enteric bacteria express type 1 fimbriae, which bind to mannose on the surface of epithelial cells in the gut (for example, enteric *E. Coli*, *Klebsiella pneumonia*, and *Salmonella*). This interaction can be assayed by agglutination of guinea pig erythrocytes (the surface of which is densely coated with mannose residues). A well-studied example is that of the uropathogenic *E. Coli*.

Certain other enteric *E. Coli* contain fimbrial hemagglutinins on their surface that bind specifically to various sialic acid-containing glycoproteins on the surface of the epithelial cell. A well-studied example of sialic acid recognition occurs on an *E. Coli* containing the K99 fimbrial lectin (M. Lindahl, R. Brossmer, T. Wadstrom, *and Glycoconj. J.* **1987**, *4*, 51-58), which recognizes *N*-glycolylneuraminic acid located specifically on NeuAc(α2,3)Gal(β1,4)Glc(β1)-ceramide. Enteric strains of *Vibrio cholera* produce a variety of hemagglutinins (R. A. Finkelstein, L. F. Hanne, *Infect. Immun.* **1982**, *36*, 1199-1208). The most prevalent of these hemagglutinins binds to fucose derivatives on the epithelial surface.

The host organism often mounts a relatively nonspecific defense that is also

polyvalent against these polyvalent pathogens. One example is the Tamm-Horsfall glycoprotein, the most abundant glycoprotein in human urine. This glycoprotein contains a variable number of N-linked oligomannose units, and binds a wide range of bacteria (F. DellOlio, F. J. J. de Kanter, D. H. van den Eijnden, F. Serafini-Cessi, *Carbohyd. Res.* **1988**, *178*, 327-332).

Most bacterial infections are also initiated by an adhesion step involving bacterial adhesions and carbohydrate determinants present on the host cell (Matrosovich. 1989. FEBS 252:1,2:1-4). One example of adhesion of a bacterium to the surface of a cell involves E. coli and urethral endothelial cells. Uropathogenic strains of the bacterium *E. coli* attach both directly and indirectly to the surface of epithelial cells in the urethra and bladder using polyvalent interactions (Figure 2). Several bacterial proteins have been identified that confer this tissue preference. Two examples are P-fimbrae (containing protein G) and type I fimbrae (containing the FimH adhesin), and both are located on the surface of *E. Coli.* We illustrate polyvalency in this system using P-fimbrae as an example. First, these uropathogenic bacteria can use the lectin-like protein G located on the tips of their P-fimbrial filaments to adhere strongly and specifically to multiple copies of the Gal(β1,4)Gal (P_K antigen) portion of a glycolipid present on the surface of the epithelial cells in the urinary tract, especially the kidney. Second, multiple copies of F-protein on the surface of the *E. coli* attach polyvalently to fibronectin, a soluble glycoprotein. The fibronectin, in turn, binds polyvalently to the surface of the epithelial cell. The *E. coli* collect in these tissues of the urinary tract, multiply there and may cause disease (especially pyelonephritis). In general, bacteria bind either directly to a cellular surface, or to molecules in the extracellular matrix of preferred tissues. Bacteria bind both to sugars and to proteins.

Table 4. Examples of Bacteria that Bind Sugars (S) and Proteins (P) on the Surfaces of Host Cells

Name of Bacterium	Disease	Molecule on Bacterium	Molecule on Host
Actinomyces naeslundii 12104 and A. viscosus LY7	Chronic oral disease		S: GalNAc beta-containing glycosphingolipids (GSLs), e.g. GalNAc(β1,3)Gal(α)-O-ethyl
Candida albicans	Part of normal fauna of the oral and (female) genital tract, and causes disease secondary to a predisposing condition (e.g. diabetes mellitus, immunodeficiency, long-dwelling catheters, antimicrobials that eliminate normal bacterial fauna)	P: 37-kDa laminin receptor P: 58-kDa fibrinogen-binding mannoprotein P: C3d receptor P: Ubiquitin	S: Fucose-containing glycosides on epithelial surface
Chlamydia trachomatis	Chronic keratoconjunctivitis that can proceed to scarring and blindness Sexually transmitted diseases Respiratory infections	S: Polysaccharide containing 7-9 mannose residues	P: Mannose binding proteins
Enteroaggregative *Escherichia coli* (*EAggEC*)	Food poisoning	P: Hemagglutinins	S: Sialic acid
Uropathogenic *Escherichia coli*	Urinary Tract Infection	P: PapG adhesin P: FimH adhesin	S: Gal(α1,4)Gal on glycolipid P?: Uroplakins Ia and Ib
S-fimbriated *Escherichia coli*	Cerebral vascular complications of meningitis		S: NeuGc(α2,3)Gal and NeuAc(α2,8)NeuAc S: NeuGc(α2,3)Gal and NeuAc(α2,8)NeuAc (Found on endothelial cells of brain micro-vasculature)
Helicobacter pylori	Gastroduodenal ulcers, gastric cancers	P: Hemagglutinin P: Laminin binding protein	S: Lewis(b) blood group antigen S: Neu5Ac(α2,3)Gal (SA dependent strains)

Mycoplasma pneumoniae	Pneumonia	P: 40 and 90 kDa protein on the tips of the pili	S: Long-chain sialo-oligosaccharides
Mycoplasma bovis	Pneumonia, genital and urinary tract infection	P: 26 kDa protein	S: Sialic acid residues and probably also sulfatide groups
Neisseria Meningococcus	Meningitis, acute adrenal insufficiency	P: Pili (unusual in that pili contain digalactosyl-2,4-diacetamido-2,4,6-trideoxyhexose)	P: CD66 on epithelial cells and neutrophils P: Glycoprotein G of RSV on surface of target cell (coinfection)
Porphyromonas (Bacteroides) gingivalis	Gingivitis		S: D-GalNAc[116]
Pseudomonas aeruginosa	Colonizes regions devoid of natural defenses (catheters), and causes disease in immunodeficient hosts	P: Pilus adhesin	S: GalNAc(β1,4) in asialo-GM1 and asialo-GM2 or GM1 S: Salivary mucin glycopeptides (sialic acid) S: Lactose of glycolipids
Rhizobium lupini		P: L-fucose binding protein	S: L-fucose
Staphylococcus saprophyticus	Urinary tract infection	P: Surface lectins	S: Blood group A (terminal GalNAc)
Streptococcus suis	Meningitis		S: Gal(α1,4)Gal present in the P1 and Pk blood group antigen S: N-acetylneuraminyl alpha 2-->3 poly-NAc-lactosamine glycans; NeuNAc(α2,3)Gal(β1,4)GlcNAc
M+ group A streptococci	Nasopharyngitis; release of erythrogenic toxins can cause scarlet fever Can occur as secondary infection to influenza virus, and ultimate cause of fatal pneumonias in these cases		P: C3, mainly C3b and iC3b on PMN

Streptococcus sanguis *Streptococcus sobrinus* (oral cavity)	Part of normal flora in the upper respiratory tract: *deficiency* can cause disease		S: Sialic acid of salivary glycoproteins
Yersinia enterocolitica	Severe abdominal pain and diarrhea; Can cause fatal sepsis in some cases		S: Gal, GalNAc, Lac in intestinal mucin

Table 5.1 Examples of Bacteria that bind to the derivatives of the glycolipid lactosylceramide

Name of Bacterium	Target Tissue
Bacteroides fragilis	Large intestine
Bacteroides ovatus	Large intestine
Bacteroides vulgatus	Large intestine
Bacteroides distasonis	Large intestine
Bacteroides thetaiotamicron	Large intestine
Lactobacillus fermentum	Large intestine
Lactobacillus acidophilus	Various places
Fusobacterium necrioohorus	Large intestine
Fusobacterium varium	Large intestine
Clostridium difficile	Large intestine
Clostridium botulinum	Large intestine
Propionibacterium granulosum	Skin, large intestine
Propionibacterium acne	Skin
Propionibacterium freudenreichii	Milk products
Actinomyces viscosus	Mouth
Actinomyces naeslundii	Mouth
Shigella dysenteriae	Large intestine
Shigella flexnerii	Large intestine
Shigella sonnei	Large intestine
Salmonella typhimurium	Large intestine
E. coli	Intestine
Vibrio cholerae	Small intestine, Large intestine
Campylobacter jejunii	Intestine
Hemophilus influenzae	Respiratory tract
Yersinia pseudotuberculosis	Intestine
Yersinia pestis	Intestine
Neisseria gonorrhoeae	Genital tract
Pseudomonas aeruginosa	Respiratory tract

Table 5.2. Examples of bacteria that bind sugars (S) and proteins (P) in the extracellular matrix

Name of Bacterium	Disease	Receptor (Ligand) on Bacterium	Ligand (Receptor) on the Host Matrix
Aspergillus fumigatus conidia	Various localized fungal infections		P: D domains of the fibrinogen molecule
Borrelia burgdorferi	Lyme disease		P: Integrin alpha IIb beta 3 (glycoprotein IIb-IIIa) (RGD)
Bordetella pertussis	Whooping cough (pertussis)	P: Pertactin and filamentous hemagglutinin (FHA); cell-binding sequence (RGD)	
Candida albicans	Part of normal flora of the oral and (female) genital tract, and causes disease secondary to a predisposing condition (e.g. diabetes mellitus, immunodeficiency, long-dwelling catheters, antimicrobials that eliminate normal bacterial fauna)	P: 37-kDa laminin receptor P: 58-kDa fibrinogen-binding mannoprotein P: C3d receptor P: Ubiquitin	S: Cell-wall polysaccharide of *Streptococcus gordonii* during coinfection S: various oligosaccharides containing both beta-1,2 and alpha-1,2 linkages
Chlamydia trachomatis	Chronic keratoconjunctivitis that can proceed to scarring and blindness Sexually transmitted diseases Respiratory infections	P: Heparin-binding protein	S: Heparan sulfate-like GAG with minimum chain length decasaccharide
Enterococcus faecalis	Diarrhea		S: Galactose, fucose, and mannosamine, but not mannose
Escherichia coli with S fimbriae	Gastrointestinal infections	P: S-fimbriae protein SfaA	S: Inhibited by NeuAc(α2,3)lactose S: Glycolipids containing terminal Gal($3SO_4$)β-1 residues
Haemophilus influenzae	Upper respiratory infections, often secondary to influenza virus	P: High-molecular-weight proteins (HMW-1 and HMW-2)	

Leishmania donovani	Protozoan parasite causing kala-azar (visceral leishmaniasis)	P: Heparin-binding protein	S: Heparin
Mycobacterium paratuberculosis	Not a major human pathogen		P: Fibronectin
Mycobacterium bovis	Chronic granulomatous infections, especially in the lung	P: Protein 85B and p55 protein	P: Collagen-binding domain of fibronectin, glycoprotein present in plasma
Mycobacterium avium-intracellulare (MAI)	A common pathogen in AIDS patients		P: Laminin, collagen I, and fibronectin
Prevotella intermedia	Periodontal infection	P: Lactoferrin-binding protein (lactoferrin inhibits the binding)	P: Fibronectin, collagen type I and type IV and laminin
Salmonella enteritidis	Severe diarrhea (common food poison from chicken and chicken eggs)		P: Fibronectin
Staphylococcus aureus	Major human pathogen responsible for wide range of infections. Can proceed to meningitis, endocarditis and osteomyelitis	P: Three repeats of a 38-residue D motif P: Fibronectin-binding proteins (galel A and ZZ-FnBP B) P: 60 kDa protein P: Collagen binding domain (CBD) of collagen adhesin P: Collagen receptor containing either two or three copies of a 187-amino-acid repeat motif	P: N-terminal 29 kDa fragment of fibronectin (Fn29K) P: Fibronectin P: Bone sialoprotein (BSP) (small (~80 kDa) integrin binding, RGD-containing bone matrix glycoprotein P: Collagen of cartilage P: Collagen P: N-terminal region (heparin binding domain) of fibronectin
	Osteomyelitis and infectious arthritis	P: Collagen adhesin P: Fibrinogen receptor (clumping factor) fibronectin-binding protein (FnBP) P: Unknown protein (60 kDa)	P: Collagen (high degree of specificity and affinity) P: Fibrinogen P: Laminin (the pepsin-derived (P1) fragment) P: Vitronectin
Staphylococcus saprophyticus	Urinary tract infections		S: Adherence is partially inhibited by mannose

Streptococcus	Wide range of gastrointestinal and respiratory infections		P: Collagen type I
Group A streptococci	Infective endocarditis, glomerulonephritis, and rheumatic fever	P: Lipoteichoic acid (LTA) and M protein (binding is inhibited by LTA) P: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) P: Protein F P: PAM (related to the M proteins) P: M3 protein	P: Fibronectin P: Human plasminogen and plasmin P: Multiple binding to plasma fibrinogen, albumin and fibronectin
Nephritis(+) and nephritis(-) group A streptococci	Glomerulonephritis	P: Nephritis plasmin binding protein (NPBP)	P: Human plasmin (the binding is blocked by ε-amino caproic acid)
Streptococcus bovis	Urinary tract infections, endocarditis	S: Lipoteichoic acid derivative	
Streptococcus defectivus	Not a human pathogen	P: Surface protein (*ca.* 200 kDa)	S: Cell-secreted extracellular matrix (ECM)
Streptococcus dysgalactiae	Neonatal sepsis and meningitis	P: Fibronectin (Fn) receptors FnBA and FnBB	P: Fibronectin
Streptococcus pneumoniae	Pneumonia		P: Laminin, collagen types I, II and IV, fibronectin, and vitronectin
Streptococcus pyogenes	Major pathogen responsible for wide range of systemic and local infections, and associated with post-streptococcal immunological disorders	P: Protein F (X-ray structure known) P: Streptococcal fibronectin-binding protein (Sfb protein, 37-amino-acid sequence) P: 9 kDa glycosaminoglycan-binding protein (GAG-BP) P: M protein	P: Fibronectin P: Basal laminae of human cardiac muscle
Veillonella atypica PK1910 (oral bacterium)	Oral infections		S: Co-aggregations with certain human oral streptococci (both lactose-inhibitable and lactose-noninhibitable)
Yersinia enterocolitica	Abdominal pain and diarrhea	P: Membrane protein YadA	P: Cartilage-derived human cellular fibronectin and human plasma fibronectin

Yersinia pseudotuberculosis	Abdominal pain and diarrhea	P: Adhesin YadA	P: β1 integrins

Another example involves the binding of cells to polyvalent molecules such as, for example, bacteria, antibodies and macrophages. All classes of antibodies -- one of the key groups of proteins making up the immune system -- have multiple equivalent receptor sites: two (IgD, IgE, IgG, IgA), four (IgA), six (IgA) or ten (IgM). Polyvalent binding to the structures that these antibodies recognize -- antigens or other ligands present on the surfaces of bacteria, viruses, parasites, drugs, "non-self" cells, or other structures including non-covalent complexes not usually present in the blood circulation -- seems to be an ubiquitous characteristic of immune recognition. These interactions may both inhibit processes important to infection (e.g., attachment of a foreign organism to target cells) and promote clearance (removal of the foreign particles either by degradation by macrophages and other components of the immune system, or by filtration by the kidney). Polyvalency is used here for high binding affinity or avidity to surfaces that have repeated epitopes, a defining characteristic of the surfaces of almost all invading pathogens.

Mannose residues on the tail (the Fc portion) of the antibody interact with mannose receptors (the Fc receptor) on the surface of a macrophage (a type of white blood cell important for clearing infectious particles). The interaction of a single Fc portion with its receptor seems to be too weak to induce a response by the macrophage; that is, free (uncomplexed) antibody in solution does not activate macrophages, nor does a single antibody bound to a degraded piece of foreign pathogen. Multiple antibodies bound to the surface of an infecting particle do, however, interact strongly with multiple receptors on the surface of the macrophage, and give a three-layered structure stabilized at both interfaces through polyvalent interactions.

In addition, not only does polyvalency in this system permit stability and specificity in the recognition of a bacterium by a macrophage, but subsequent action by the macrophage is also critically dependent on polyvalent interactions. Multiple interactions between the macrophage and the antibody-coated bacterium lead to a cross-linking of the surface receptors on the macrophage, triggering an internal signal in the macrophage to ingest (phagocytose) the bacterium, leading to its degradation.

Other examples of polyvalent molecules attaching to the surfaces of cells are given in Tables 6 and 7.

Table 6. Receptors for Bacterial Toxins

Bacterium	Toxin	Ligand
Vibrio cholerae	Cholera toxin	**S:** GM1: Galβ1,3)GalNAc(β1,4)(NeuAc(α2,3))Gal(β1,4)Glc(β)Ceramide **S: Isoligands** NeuAc(α2,3)Gal(β1,3)GalNAc(β)(NeuAc(α2,3)Gal(β1,4)Glc(β)Ceramide, Gal(β)GalNAc(β1,4)(NeuAc(α2,8)NeuAc(α2,3)Gal(β1,4)Glc(β)Ceramide, GalNAc(β1,4)Gal(β1,3)GalNAc(β1,4)((NeuAc(α2,3)Gal(β1,4)Glc(β)Ceramide, Fuc(α1,2)Gal(β1,3)GalNAc(β1,4)((NeuAc(α2,3)Gal(β1,4)Glc(β)Ceramide, Gal(β1,3)GalNAc(β1,4)(R-NeuAc(α2,3)Gal(β1,4)Glc(β)Ceramide: R=Lucifer yellow CH, Rhodamine, or DNP
E. coli	Heat-labile toxin	**S:** GM1
Clostridium tetani	Tetanus toxin	**S:** Gal(β1,3)GalNAc(β1,4)((NeuAc(a2,8))NeuAc(2,3)Gal(β1,4)Glc(β)Ceramide **S: Isoligands** NeuAc(α2,3)Gal(β1,3)GalNAc(β1,4)((NeuAc(α2,8))NeuAc(α2,3)Gal(β1,4)Glc(β)Ceramide, NeuAc(α2,8)NeuAc(α2,3)Gal(β1,3)GalNAc(β1,4)(NeuAc(α2,8)NeuAc(α2,3)Gal(β1,4) Glc(β)Ceramide
Clostridium botulinum	Botulinum toxin A and E	**S:** NeuAc(α2,8)NeuAc(α2,3)Gal(β1,3)GalNAc(β1,4)(NeuAc(α2,8))NeuAc(α2,3)Gal(β1,4) Glc(β)Ceramide
Clostridium botulinum	Botulinum toxin B, C, and F	**S:** NeuAc(α2,3)Gal(β1,3)GalNAc(β1,4)(NeuAc(α2,8))NeuAc(α2,3)Gal(β1,4)Glc(β)Ceramide
Clostridium botulinum	Botulinum toxin B	**S:** Gal(β)Ceramide
Clostridium perfringens	Delta toxin	**S:** GalNAc(β1,4)(NeuAc(α2,3))Gal(β1,4)Glc(β)Ceramide
Clostridium difficile	Toxin A Shiga-like toxin (SLT)-I and SLT-II/IIc	**S:** Gal(α1,3)Gal(β1,4)GlcNAc(β1,3)Gal(β1,4)Glc(β)Ceramide **S:** Gal(α1,4)Gal(β) (P1 disaccharide), Gal(α1,4)Gal(β1,4)GlcNAc(β) (P1 trisaccharide), or Gal(α1,4)Gal(β1,4)Glc(β) (Pk trisaccharide)

Shigella dysenteriae or E. coli	Shiga toxin Vero toxin	S: Gal(α1,4)Gal(β)Ceramide S: Gal(α1,4)Gal(β1,4)Glc(β)Ceramide
Bordella pertussis	Pertussis toxin	S: NeuAc(α2,6)Gal
S. Dysenteriae I	Dysenteriae toxin	S: GlcNAc(β1)

Table 7. Examples of cross-linking of surface receptors as a mechanism of signal transduction

Process	Receptor -- Ligand
Degranulation of mast cells (Allergy)	Allergens oligomerize IgE on the surface of the mast cell (P, N:N)

Cellular differentiation and growth (Developmental Biology: Differentiation, Migration, Apoptosis)	Fibroblast Growth Factor (FGF) dimerizes FGF receptor. A role for heparin in forming a polyvalent template for FGF and other heparin-binding growth factors is emerging. (P, N:N)
	Epidermal growth factor (EGF) dimerizes EGF receptor. (NP)
	Cytokine Stem Cell Factor (SCF) dimerizes Kit receptor on surface of Stem Cell. (P, 1:2)
	Transforming Growth Factor (TGF) dimerizes TGF receptor. (NP)
	Platelet-derived growth factor (PDGF) dimerizes PDGF receptor. (P, 1:2)
	A single Human Growth Hormone (hGH) dimerizes hGH receptor. (P, 1:2)
	Hepatocyte Growth Factor (HGF) dimerizes c-Met receptor. Heparin may provide a polyvalent template for HGF (see FGF above). (P, N:N)
	EPO dimerizes EPO receptor on erythrocyte precursor cell. (P, N:N)
	Vascular Endothelial Growth Factor (VEGF) binds to VEGF Receptor Flt-1. (NP)
	IL6 hexamerizes IL6 receptor. (P)
	IL4 dimerizes its receptor. (P, 1:2)
	Human Macrophage Colony-Stimulating Factor (M-CSF) dimerizes M-CSF receptor. (P, 1:2)
	Lactogenic hormone can dimerize prolactin receptors (PRLR). (P, 1:2)
	Two monovalent granulocyte-colony stimulating factors (G-CSF) stimulate the dimerization of two G-CSF receptors. (NP)
Compaction during early embryogenesis	Receptor for X hapten and multivalent X hapten. (Free multivalent versions of X hapten decompact. Monovalent X haptens have no effect).(P, N:N)
Parasympathetic Response (Autonomic Nervous System)	Acetylcholine receptor heterodimerization by unknown mechanism. (NP?)

Table 7. (continued)

Acrosomal reaction in the interaction of sperm with egg (Reproductive Biology)	GalTase on sperm -- GlcNAc on the exterior of the egg. (P, N:N)
Peptide-independent clonal expansion of B-cells (Immunology)	Antibody on the surface of a B-cell -- Polysaccharide coats on the surface of microorganisms. (P, N:N)
Interferon actions	Interferon-alpha (IFN-alpha) dimerizes its receptor. (P, N:N)
T-cell clonal expansion	T lymphocyte receptors CD28 and CTLA-4 bind and are oligomerized by co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) on antigen presenting cells. (P, N:N)
Bacterial chemotaxis	Aspartate dimerizes bacterial receptor Tar. (NP)

P, 1:2 = Heterobivalent ligand dimerizes two surface receptors; P, N:N = N-valent ligand oligomerizes N surface receptors; P = probably polyvalent, but with unknown ligand-receptor stoichiometry; NP = Possibly a non-polyvalent mechanism.

Polyvalency also may be important in preventing undesired interaction, i.e., with polyvalent inhibitors. Where polyvalency can strongly promote desired interactions, it can also be a useful strategy in preventing certain undesired biological interactions, especially those that are themselves polyvalent (here, polyvalency is used against polyvalency). For example, the fluid coating the interior of the lungs of most mammals contains several mucins (proteins presenting oligosaccharides terminated in sialic acid, SA). These mucins, especially ?2-macroglobulin, can bind to influenza and other SA-binding viruses, and thereby inhibit their attachment to target cells.

In the selection of groups A for polyvalent presentation, the use of groups A which interact with the pathogenic particle are preferred over those that interact with the host. In one embodiment, the subject presenters are used to block cell-bacteria interactions. In another embodiment, the subject presenters are used to block cell-fungus interactions. In yet another embodiment, the subject presenters are use to block cell-virus interactions. In a further embodiment, the subject presenters are used to block cell-parasite interactions. For example, the binding of *Entamoeba histolytica* trophozoites to host galactose (Gal) and N-acetylgalactosamine (GalNac) residues. (Adler *et al.* 1995. J. Biol. Chem. 270:5164). In some embodiments, e.g., gene therapy applications, it may be desirable to promote cell-pathogen, e.g., cell-virus, interactions.

In another embodiment, polyvalent presenters of the present invention can be made which modulate pathogen-extracellular matrix interactions. For example, polyvalent presenters that prevent the interaction of bacteria or fungi with extracellular matrix can be inhibited. In another embodiment, the subject presenters can be used to modulate cell-extracellular matrix interactions.

In yet another embodiment, polyvalent presenters that modulate pathogen-pathogen interactions can be constructed. Such presenters will be useful in the treatment of, e.g., infectious states which result from the use of indwelling devices, such as prostheses and catheters, or in the disruption of biofilms *ex vivo*.

In yet another embodiment, cell-toxin interactions may be modulated. For example, shiga toxin from *Shigella dysenteriae* type 1 binds to cellular glycoproteins or glycolipids having the galabiose disaccharide (Galα1-4Galβ) determinant. Polyvalent presenters of the present invention can be made to present groups A which can block any

such cell-toxin interaction. In another example, a polyvalent presenter that reacts with a hemorrhagic toxin, such as that produced by *Croatalus viridis viridis* can be made.

In another embodiment the subject presenters can be used to modulate cellular responses which are associated with polyvalency. For example, cytokine production by cells can be modulated by replacing a stimulator cell in a cell-cell interaction that normally leads to cytokine secretion. For example, the L-selectin ligands on tumor cells have been shown to stimulate monocytes and macrophages to produce tumor necrosis factor. Sialyl Lewis x (sLe^x) tetrasaccharide has been proposed to function as an HEV ligand for L-selectin, but the natural ligand may be more complex (Putz and Mannel. 1996. Scand. J. Immunol. 44:556-564). Polyvalent presenters bearing A groups which mimic the tumor cell groups recognized by mononuclear cells could be used to mimic this response. Likewise, presenters bearing A groups which block this reaction could be used to inhibit this response. In other embodiments, other states associated with polyvalency in which a polyvalent presenter could be used include T cell cytokine production, mast cell and/or basophil degranulation, lymphocyte selection, and T or B cell apoptosis (Seledtsov and Seledtsova. 1995. Biomed & Pharmacother. 1996. 50:170), all of which are states associated with polyvalency.

Treatment of diseases or conditions, e.g., associated with polyvalency

In addition to targeting any of the specific biological events that are associated with the disease or condition, the skilled artisan could design polyvalent presenters that target specific diseases or conditions or manifestations of those events presented by subjects. The present invention is intended to encompass the use of all groups presented polyvalently or drugs provided as part of a polyvalent presenter of the present invention. See e.g., Harrison's Principles of Internal Medicine, Thirteenth Edition, Eds. T.R. Harrison *et al.* McGraw-Hill N.Y., NY; Goodman and Gilman's Pharmacological Basis of Therapeutics, 9th Ed. Eds. Joel GG Hardman, Alfred Gilman, Lee L. Limbird, New York. McGraw Hill 1995; and the Physicians Desk Reference 50th Edition 1997. Oradell New Jersey, Medical Economics Co. The complete contents of each of these texts are expressly incorporated by reference herein. The content of their teachings with regard to disease, or conditions and the teachings with respect to possible drugs, e.g., groups A are even more particularly incorporated by reference herein. For example, polyvalent presenters can be

designed to modulate infectious disease, including e.g., inhibiting host-parasite interactions, augmenting immunization or vaccination strategies, and reducing sepsis or septic shock. More specifically, the subject presenters will be useful in the treatment of, *inter alia*: infectious diseases of the upper respiratory tract; infective endocarditis; intraabdominal infections and abscesses; acute infectious diarrhea diseases and bacterial food poisoning; sexually transmitted diseases; pelvic inflammatory disease; urinary tract infections and pyelonephritis; infectious arthritis; osteomyelitis and infections of prosthetic joints; infections of the skin, muscle, and soft tissues; infections in injection drug users; infections from bites, scratches, burns, and environmental organisms; and nosocomial infections. Accordingly, the subject presenters will be useful in the treatment of states caused by gram positive organisms (e.g., pneumococcal infections, staphylococcal infections, streptococcal infections, corynebacterial infections, listeria infections, tetanus, botulism clostridial infections, and anthrax), and states caused by gram negative bacteria (e.g., meningococcal infections, gonoccoccal infections, *Moraxella* and *Kingella* infections, *Haemophilus* infections, *Legionaella* infections, pertussis, infections with enteric bacilli, *Pseudomonas* infections, salmonellosis, shigellosis, *Campylobacter* infections, cholera and other vibrioses, brucellosis, tularemia, *Yersinia* infections, bartonellosis, and donovanosis. The subject presenters will also be useful in the treatment of nocardiosis, actinomycosis, mixed anaerobe infections, and mycobacterial and spiral bacterial (*H. pylori*) infections (e.g., tuberculosis, leprosy, and *Mycobacterium avium* infection). Polyvalent presenters can also be used in the treatment of spirochetal diseases (e.g., syphilis, treponematoses, leptospirosis, relapsing fever, and lyme borreliosis). *Rickettsia*, *Mycoplasma*, and *Chlamydia* infections will, likewise, benefit from treatment with the subject polyvalent presenters.

Viral infections will also benefit by treatment with the subject polyvalent presenters. For example, DNA viruses (e.g., herpes simplex, varicella-zoster, Epstein-Barr, cytomegalovirus infection, poxvirus infection, parvovirus, and human papillomavirus) and RNA viruses (e.g., retroviruses, influenza, gastroenteritis, enteroviruses and reoviruses, rubeola, rubella, mumps, rabies, rhabdoviruses, and marburg-like agents, arbovirus infections, and arenavirus infections) can also be treated.

In other embodiments, the subject presenters can be used to treat fungal infections, e.g., histoplasmosis, coccidioidomycosis and paracoccidioidomycosis, blastomycosis, cryptococcosis, candidiasis, aspergillosis, mucormycosis, among others can be

treated. In other embodiments, polyvalent presenters can be used to treat protozoal infections (e.g., amebiasis, malaria and babesiiosis, leishmaniasis, trypanosomiasis, toxoplasma, pneumocyctis cariniii, giardiasis, cryptosporidiosis, and trichomoniasis). In still other embodiments, the subject presenters can be used to treat helminthic infections (e.g., trichinosis, tissue nematodes, intestinal nematodes, filariasis, loiasis, onchocerciasis, dracunculiasis, schistosomiasis and other trematode infections, or cestodes). In still other embodiments, the subject presenters can be used to treat ecotparasite infestations.

In still other embodiments the subject presenters are also useful in modulating the immune response, both by upregulating and downregulating that response. Accordingly, the subject polyvalent presenters will be useful both in the treatment of immunodeficiency diseases, (regardless of the underlying cause) as well as in the treatment of autoimmune disease and resulting immune-mediated injury. In certain embodiments the subject presenters will be useful in inhibiting graft rejection.

In other embodiments, the subject polyvalent presenters are also useful in the treatment of disorders of coagulation and thrombosis and in anticoagulant, fibrinolytic, and antiplatelet therapy.

In still other embodiments, the subject presenters are also useful in the treatment of neoplasia. In preferred embodiments, the subject presenters are used to inhibit metastasis of primary tumors.

The initial event in the biological action of certain plant-derived and bacterial toxins involves their multivalent adhesion to the surface of a cell; that is, their binding by the simultaneous interaction of multiple toxin receptors to multiple cellular ligands (Lord, J. M.; Roberts, L. M.; Robertus, J. D. *FASEB* **1994**, *8*, 201-208; Montecucco, C.; Papini, E.; Schiavo, G. *Experientia* **1996**, *52*, 1026-1032; Kuziemko, G. M.; Stroh, M.; Stevens, R. C. *Biochem.* **1996**, *35*, 6375-6384; Richardson, J. M.; Evans, P. D.; Homans, S. W.; Donohue-Rolfe, A. *Nature Struct. Biol.* **1997**, *4*, 190-193. In one approach polyvalent polymeric galactosides can be used to block the adhesion of the cytotoxic lectins from *Ricinus communis*, commonly referred to as ricins, to the surface of mammalian cells. These polymers act by preventing the ricins from interacting with galactoside residues on the cell surface. The number of receptor-ligand interactions involved in ricin-cell adhesion is small.

Ricins, *Ricinus communis* agglutinins (RCA_{60}, RCA_{120}) belong to a

class of cytotoxic lectins, referred to as ribosomal-inactivating proteins (RIPs). One ricin subtype, RCA_{60} (an A_1B_1 covalent complex; MW ~ 65 kDa) is composed of two subunits: an A-chain—an *N*-glycosidase that cleaves a specific adenine base of ribosomal RNA hydrolytically and inhibits protein synthesis—and a B-chain—a sugar-binding lectin (Kuziemko, G. M.; Stroh, M.; Stevens, R. C. *Biochem.* 1996, *35*, 6375-6384) See also Baenziger, J. U.; Fiete, D. *J. Biol. Chem.* 1979, *254*, 9795-9799; Houston, L. L.; Dooley, T. P. *J. Biol. Chem.* 1982, *257*, 4147-4151; Rivera-Sagredo, A.; Solis, D.; Diaz-Mauriño, T.; Jimenez-Barbero, J.; Martin-Lomas, M. *Eur. J. Biochem.* 1991, *197*, 217-228. A second ricin subtype, RCA_{120} (2 x $A'_1B'_1$; MW ~ 130 kDa) is related closely to RCA_{60} in many aspects of its structure and function; it is composed of two, non-covalently associated $A'_1B'_1$ units (Endo, Y.; Mitsui, K.; Motiguki, M.; Tsurugi, K. *J. Biol. Chem.* 1987, *262*, 5908-5912). The A-chain-catalyzed inactivation of rRNA is the major cause of the toxicity of ricins. This action cannot, however, occur without cellular internalization of the toxins; this internalization occurs via receptor (B-chain)-mediated endocytosis.

Ricins inhibit biosynthesis of proteins strongly in mammalian cells. The molecular action of ricins requires cellular internalization, which begins with adhesion of ricin to the surface of the cell: this strong toxin-cell attachment occurs by the simultaneous binding of multiple galactoside-recognition sites of ricin to multiple galactoside (Gal) groups of cell-surface glycoproteins and glycolipids.

Ricins attach to the cellular surface using multivalent, selective binding of Gal-binding sites of the B chain (~3 sites per B chain) to multiple β-D-galactose (β-Gal)- and β-*N*-acetyl-D-galactosamine (β-GalNAc)-terminated oligosaccharides present on the surface of cells (Roberts, L. M.; Lamb, F. I.; Pappin, D. J. C.; Lord, J. M. *J. Biol. Chem.* 1985, *260*, 15682-15686) (The number of Gal-binding sites per B subunit has been controversial. A number of studies suggested that RCA_{60} and RCA_{120} have ~ 3 (> 2) and ~ 6 (> 4) Gal-sites, respectively). Binding studies of ^{125}I-labeled ricins indicated that ricins (and the isolated B-chain) attached to human erythrocytes with dissociation constant K_d ~ 0.1-0.01 μM. Simple monovalent analogs of β-D-galactosides have relatively low affinity for RCA_{120}, RCA_{60} and the isolated B-chain, when studied by various techniques (equilibrium dialysis,

fluorescence, and NMR) ($K_d \sim 10^3$–10^2 μM). As a result of the present invention polyvalent presenters can be produced to achieve relatively tight binding of ricins to the surface of the cell.

Ricins are, in many aspects of structure and modes of action, related closely to bacterial toxins (anthrax; cholera; shiga; verotoxin) and other plant-derived cytotoxic lectins (ribosomal-inactivating proteins; abrin). These toxins are the objects of current research as the cytotoxic components of immunotoxins in development as anticancer drugs, and have been classified as threat agents in biological warfare.

In further embodiments, therefore, the subject presenters are useful in preventing adhesion of ricins to erythrocytes. In this regard the polyvalent presenters may be used to block the toxicity of ricins based on polyvalency by blocking ricin-cell adhesion. In this manner, the first step in their entry into the cell is blocked, using polyvalent polymeric inhibitors capable of competing with the multiple copies of the ligands presented on the surface of the cell. The synthesis and bioassay of polymeric polyvalent molecules designed to inhibit adhesion of ricins (RCA_{120} and RCA_{60}) to chicken red blood cells are discussed herein. The inhibitors are derivatives of poly(acrylic acid) and poly(butadiene-*co*-maleic acid); they present multiple copies of galactoside-containing side chains as amide groups: pAA(Gal) and pBMA(Gal). One of the inhibitors, pAA(Gal-βO-L_1; $\chi^{Gal} = 0.4$) showed an inhibitory activity, $K_i^{HAI} =$ 0.14 μM (calculated on a per galactoside basis) against RCA_{120}; this polymeric polyvalent galactoside is ~ 1500 times more active than monovalent Gal-β-OMe, and 270 times more active than monovalent Gal-βO-L_1NH_2. The polymeric polyvalent inhibitors are significantly more active in blocking hemagglutination induced by RCA_{120} (with ~ 6 Gal receptor sites) than that by RCA_{60} (with ~ 3 Gal receptor sites). The molecular basis of the enhanced activities of the polymeric polyvalent galactosides in blocking hemagglutination is ascribed to multivalent (that is, multiple, simultaneous) interactions of the Gal receptor sites of ricin and Gal moieties on the polymers. We have demonstrated that synthetic glycopolymers can inhibit ricin-cell adhesion. These polymers are, thus, a new class of synthetic antitoxins. Although the action of these polymers may mimic the action of anti-toxin antibodies by preventing the approach of the ricin Gal-binding site to Gal moieties on the surface of the cell, they also introduce

the possibility of fundamentally new mechanisms of inhibition: entropically-enhanced occupancy of the ricin Gal-binding sites; steric stabilization of unoccupied Gal binding sites by the surrounding polymer. The observation that these polymers are more active against RCA_{120} than against RCA_{60} suggests that polyvalent inhibitors are more active against receptors with larger number of ligand-binding sites than those presenting fewer.

A scheme summarizing ricin-cell interactions (for both RCA_{120} and RCA_{60}), and a strategy for the neutralization of ricins using a polymeric polyvalent agent is set forth in Figure 3 and involves the following: (a) Receptor-ligand mediated adsorption of ricins to a mammalian cell: multivalent binding of receptor sites (B-chain) of ricins to multiple, specific cellular ligands (β-galactosides) results in tight toxin-cell association; the attachment of ricins to the cell constitutes an initial step in their toxic action. (b) The mechanism of toxicity of ricins: after entry of toxins into the interior of the cell (via endocytosis), and reductive cleavage of the A-chain from the B-chain, monomeric A-chain efficiently catalyzes hydrolytic *N*-glycosidation of a specific adenine base of ribosomal RNA; this damage interferes with protein biosynthesis. (c) Blocking of the cellular entry of ricins by a polymeric polyvalent galactoside.

Therefore, synthetic Gal-presenting polymers of the invention may be used as inhibitors that prevent the attachment of ricins to mammalian cells; these compounds, or derivatives of these compounds, are potential leads as therapeutics and as protective materials against other exposures to ricins.

Multivalent binding of toxin receptors to specific cell-surface ligands is also important in other situations. For example, abrins contain multiple receptor sites per B-chain of toxin molecule; this toxin binds selectively to galactoside residues (Lord, J. M.; Roberts, L. M.; Robertus, J. D. *FASEB* **1994**, *8*, 201-208; Sandvig, K.; Olsnes, S.; Pihl, A. *J. Biol. Chem.* **1976**, *251*, 3977-3984). Cholera toxin utilizes pentameric B (sugar-binding) subunits, each of which recognizes GM_1 pentasaccharide (Gal(β1,3)GalNAc(β1,4)(NeuAc(α2,3))Gal(β1,4)Glc(β1,1)-ceramide) residues on the cellular surface (Kuziemko, G. M.; Stroh, M.; Stevens, R. C. *Biochem.* **1996**, *35*, 6375-6384), while shiga toxin and verotoxin utilize five B-subunits for binding to Gal-containing glycosphingolipid (globoside, Gal(α1,4)Gal(β1,4)Glc(β1,1)-ceramide) (Richardson, J. M.; Evans, P. D.; Homans, S.

W.; Donohue-Rolfe, A. *Nature Struct. Biol.* **1997**, *4*, 190-193).

Thus, the present invention finds use in designing inhibitors from pathogen (cell)-cell association (characterized by high valency: about 100-1000 sites are potentially available for interaction) to toxin-cell interactions (characterized by low valency: that is, ≤ 10 Gal receptor sites are involved).

The subject polyvalent presenters can be used to inhibit egg-sperm interactions such that fertilization is inhibited, e.g. the acrosomal reaction can be interfered with using a polyvalent presenter. Thus, the subject presenters are also useful in preventing conception, e.g., by inhibiting the sperm-egg interaction, e.g., inhibition of attachment of sperm to eggs or by inducing the acrosomal reaction prior to interaction between sperm and egg, e.g., premature activation of the reaction.

Fertilization is initiated by species-specific adhesion of sperm to the extracellular coat of egg. The sperm-egg interaction causes the sperm to undergo a series of acrosomal reactions (acrosomal process): the early reaction appears as exocytosis of acrosomal vesicles of sperm to egg coat. This acrosomal reaction leads eventually to fusion of the plasma membranes of sperm and egg, and to entry of a nucleus of the sperm into the cytoplasm of the egg.

In mouse sperm, a surface protein β-1,4-galactosyltransferase (Gal-transferase) on the plasma membrane of the sperm is the receptor that recognizes specifically GlcNAc residues of zona pellucida (ZP) glycoproteins of egg coat (Figure 4). Referring to Figure 4, a scheme is depicted summarizing an approach for induction of acrosome reaction of sperm as well as for inhibition of sperm-egg binding using a polymeric polyvalent agent, pAA(GlcNAc). (a, b) Adhesion of a mouse sperm to an egg is mediated by receptor-ligand interactions: Gal-transferase on the surface of sperm head and terminal *N*-acetylglucosamine (GlcNAc) residues of ZP3 glycoprotein on zona pellucida (ZP) of egg coat: (a) Multivalent binding of the Gal-transferase receptors to multiple GlcNAc ligands of ZP3 results in aggregation of the Gal-transferase, and (b) subsequently in induction of acrosomal exocytosis of sperm via intracellular signal transduction. (c, d) The proposed mechanism for induction of acrosome reaction and for inhibition of sperm-egg attachment by poly(acrylic acid) presenting multiple copies of GlcNAc as amide side chains, or pAA(GlcNAc). The depiction of Figure 4 is not to scale.

Of various isoforms of ZP glycoproteins of the zona pellucida, ZP3 is believed to be a glycoprotein ligand for Gal-transferase that mediates sperm-egg adhesion. The ZP3 contains a variety of multiple copies of *O*-linked *N*-acetylglucosamine (GlcNAc) moieties that are connected covalently to serine and threonine residues of the glycoprotein. Clusters of GlcNAc residues from multiple ZP3 are able to bind simultaneously to multiple surface Gal-transferase receptors of sperm head.

The acrosomal reaction of sperm occurs through an intracellular signal transduction mechanism, triggered by activation of certain G-protein coupled receptors. The activation of the G-protein receptors requires spatial aggregation of Gal-transferase (Figure 4). In the normal process of sperm-egg fertilization, ZP3 glycoproteins in ZP of the egg coat bind to and aggregate Gal-transferases: the aggregation is a result of multivalent interactions of multiple GlcNAc ligands of ZP3 and sperm surface Gal-transferases.

In the present invention polyvalent forms of GlcNAc—a mimic of multivalent GlcNAc of ZP3—induce acrosomal reaction of sperm (Figure 4). Polymeric polyvalent GlcNAc induces acrosomal exocytosis of mouse sperm, and furthermore inhibits the binding of sperm to egg in vitro.

In preferred embodiments, the polyvalent presenters of the present invention may be combined with other methods of contraception or can be used to also prevent disease while being used in the prevention of contraception. For example, the groups A can be ligands useful for treating herpes, HIV, chlamydia, human papillomavirus, gonorrhea and syphilis.

In still further embodiments, the subject presenters are also useful for treating or modulating cardiogenic shock, angina, thrombus formation within the circulatory system, heart failure, arteriosclerosis, hypertension, stroke, restenosis, failure of the kidney to secrete erythropoeitin, secretory disease, allergic reaction, psoriasis, sickle cell anemia, secondary diseases of the mematopoetic system secondary to treatment (chemotherapy, side effect of a drug) or another disease (metastasis, autoimmune disease), renal cancer, prostate cancer, brain cancer, lung cancer, thyroid cancer, colorectal cancer, stomach cancer, liver cancer, pancreatic cancer, tumor resistance to chemotherapy, adult onset diabetes, growth deficiencies, CNS trauma, neurodegenerative disease, schizophrenia, migraines, anxiety, depression, obsessive compulsive disorders, idiopathic disease, (diseases whose causes are unknown but have etiologies which are amenable to polyvalent drugs including fever of

unknown origin, multi-organ failure), ophthalmic indications - (glaucoma, myopia, retina degeneration, presbyopia), menopause, pregnancy diseases (hypertension, toxemia, diabetes and agents to assist in carrying to term, agents to assist in delivery), anesthesia, fertility, wound healing, incontinence, dermatology (acne, dandruff, uticaria), adverse reactions to drugs, and reversal of toxic effects of drugs. Even further embodiments include a condition or disease involving the modulation, e.g., promotion, of cell death, the modulation, e.g., promotion, inhibition, or augmentation, of a metabolic pathway (e.g., via an enzyme) or the targeting of a particular receptor type, e.g., clustered, seven member transmembrane receptor, or an enzyme, e.g., intracellular, extracellular, or membrane bound.

ASSAYS FOR IDENTIFYING AND TESTING POLYVALENT PRESENTERS

The present invention also provides for methods of assaying for polyvalent presenters which can be used, e.g., in identifying desirable useful groups A or in testing the efficacy of presenters. Such assays can be either *in vitro* or *in vivo*. In addition, in vitro assays can be designed which test for the ability of a polyvalent presenter to modulate the interaction between A and B, or a biological response which results from the interaction of A with B (e.g., cell adhesion assays, agglutination assays, platelet aggregation assays, ELISA assays, as well as muscle contractility assays, infectivity assays, or lymphocyte stimulation assays, and the like).

In vitro screening.

In certain embodiments, in vitro assays can be used to test the effectiveness of polyvalent presenters which assay the ability of a polyvalent presenter to interact with target binding sites B or to inhibit the interaction of Groups C with binding sites B. For example, assays which test the ability of Group A of a presenter to interact with binding sites B on a polyvalent surface (e.g., panning assays) can be used in screening presenters (Charych, D. *et al. Chem. & Biol.* 1996, 3, 113- 120).

In another exemplary embodiment, capillary electrophoresis (CE) can be used. CE is a convenient high resolution analytical technique requiring only femtomoles of material. CE allows separation of mixtures of molecules (ions, small molecules, polymers, proteins, micelles) on the basis of their charge and hydrodynamic drag. By adding groups A to the buffer solution at varying concentration, and by monitoring the influence of this concentration of the mobility of injected binding sites B, it is possible to quantitate accurately the binding constant of the group for the binding site. This technique is referred to as affinity capillary electrophoresis (ACE). For example, the affinity of a presenter for a whole virus expressing binding sites B can be determined using ACE. It has also been shown that ACE forms the basis of a very efficient library search. CE is also useful, as are GPC and light scattering, in the analysis of presenters, especially when they carry charge.

Surface Plasmon Resonance Spectroscopy can also be used (see e.g., Mrksich, M.; *et al. Langmair* 1995, 4383; Mrksich, M. *et al.* J. *Am. Chem.Soc.* 1995,117:12009; Sigal, G. B. *et al. Anal. Chem.* 1996, 68: 490) Surface plasmon resonance (SPR) has been used to study binding events at surfaces.

Model Surfaces Based on Self-Assembled Monolayers (SAM's) can also be used to assay the subject presenter molecules. SAM's of alkanethiolates on gold and silver is another model system for studying adsorption, or other molecular events occurring at interfaces. (See e.g., Mrksich, M.; Whitesides, G. M. *Ann. Rev. Biophys. Biomol. Struct.* 1996, 25:55; Whitesides, G. M. and Gorman, C. B. *Self-Assembled Monolayers: Models for Organic Surface Chemistry;* CRC Press: Boca Raton, 1995; Mrksich, M. *et al.* J. *Am. Chem.Soc.* 1995,117:12009; Sigal, G. B, *et al., Anal. Chem.* **1996,** 68: 490; Lopez, *et al.*, J. *Am. Chem. Soc. 1993, 115:*5877).

In another embodiment, agglutination can be tested, for example, using synthetic beads mixed with pathogens or cells (equivalent to hemagglutination inhibition

assays) for the quantitative analysis of polyvalent molecules. Agglutination is a highly convenient method of probing cell-cell and pathogen-cell contacts and 96-well microtiter plates are an especially suitable format for such an assay. Automation of this assay is also possible. For example, a bead can be constructed that presents a group that interacts specifically with the surface of the pathogen or cell. The construction of such a bead can consider steric access to the group relative to the background. In addition, appropriate attachment point to the group can be considered and may be based on crystal structures where available. In addition, the importance of eliminating or reducing non-specific interactions through the use of an oligoethyleneglycol background, and the importance of the surface density of the group can also be considered. Alternatively, a target cell (or surrogate target cell) can be used in certain embodiments. For example, influenza virus binds to erythrocytes, and erythrocytes, rather than beads, can be used.

In addition, relative and absolute concentrations of bead presenting groups A and the surface presenting binding sites B which cause the mixture to form a "gel" (i.e., to agglutinate) can be determined. Colored beads may be used to ease visualization. Polyvalent materials can be constructed that contain varying mole fractions of the group on the bead, or of derivatives of the group, or entirely different groups (in the latter case, inhibition can depend on steric stabilization). In general, the potency of the polyvalent material in the assay will be dependent on the mole fraction of momomeric units of the polymer that are connected to active groups. The mole fraction at which potency is maximum is expected to be system-dependent.

These same polyvalent materials can be modified so that various other groups are incorporated into the available positions on the carrier. The structure and mole fraction of various "auxiliary" (e.g., A_2- A_n) groups may increase the specificity and potency of the molecule. One mechanism whereby such enhancement is possible is by randomly locating hydrophobic pockets on the surface of the pathogen (or cell) using a small mole fraction of short hydrophobic side chains. Important properties of the polymer that are expected to control its potency include: mole fraction of active group, charge, hydrophobicity, persistence length, randomness, physical dimensions, and number of associated water molecules.

Inhibition assays can also be used, such as those that measure the extent to which a molecule prevents a biological surface, e.g., a virus, from binding to another

biological surface, e.g., a cell. The molecule may do so by binding competitively to the receptor, and prevent binding of the surface bound groups to the same binding site. One exemplary assay is the hemagglutination inhibition (HAI) assay, which is described in more detail in the appended examples and appendices. The HAI assay is based on molecules inhibiting viral agglutination (gel formation) of a solution of erythrocytes. The lower limit of effectiveness that HAI can conveniently measure is ~ 1 nM.

Another exemplary assay is the optical collision (OPTOCOL) assay, and is based on the inhibition of attachment upon collision of one erythrocytes and one virus-coated microsphere in the presence of inhibitor. This assay is performed under an inverted microscope using parallel optical tweezers, with each of the tweezers holding one of the two colliding species. The OPTOCOL assay allows quantitation of effectiveness of inhibitors that are active at << 1 nM.

Both the HAI and OPTOCOL assays yield a concentration at which the inhibition is partially effective, i.e., a concentration at which approximately half the interactions between groups A and binding sites B are inhibited. In the HAI assay, the inhibition constant is KHA or KOPTOCOL. When measuring inhibition constants whose values are reliably measurable using each assay, KHA~ =KOPTOCOL. Both these inhibition constants can be referred to as KINH.

OPTOCOL is based on the manipulation of biological particles using parallel dual optical tweezers. (Mammen, *et al.*, 1996 Chem. Biol. 3:757) This technique enables study of interactions between, e.g., a single erythrocyte and a single microsphere presenting influenza virus. This technique is especially useful for very tight-binding systems and can also be used for studying the mechanisms of polyvalent inhibition.

Most preferred assays weight the characteristics of the polyvalent group that are important for achieving the desired goal. For example, an effective polyvalent molecule for the purpose of aggregating a polyvalent binding site might be composed of "n" greater than 10 groups linked through a long flexible linker. The assay, therefore, must reflect the ultimate purpose of the polyvalent species.

A number of methods for probing, assessing and quantitating polyvalent interactions exist. Some assays may be direct measures of affinity; from these affinities, one may extract free energies of interaction. Other assays may measure a complex aggregate of characteristics, only one of which is free energy of interaction. These other characteristics

may include extent of hydration, ability to stabilize a molecule or surface sterically, and/or ability to crosslink multivalent receptors.

To quantify a binding constant thermodynamically (i.e., to obtain a binding constant), the relative proportions of uncomplexed and complexed group (or binding site) must be measured (directly or indirectly). Depending on the stability of the complex (related to its lifetime), different techniques can be used. .

Aggregation assays can be used to measure the ability of a polyvalent group to aggregate a polyvalent binding site (precipitation, gel formation, aggregation). For example, a polyvalent presenter can precipitate polyvalent binding sites B on a surface in immunoprecipitation assays. Although the affinity of the polyvalent entity is important in determining the ability of the polyvalent presenter to precipitate, other characteristics can be important. For example, at low concentrations, the presenter may not bind to polyB; at some optimal concentration zone, precipitation occurs; while at higher concentrations, each binding site B is bound by a group A and precipitation again does not occur. In this example, which is analogous to antibody precipitation reactions, affinity alone does not determine the pattern of precipitation.

In vivo assays

In *vivo* assays can be conducted which, for example, measure the polyvalent presenter therapeutic effect for a disease or condition in an animal, for example, protection against infection could be measured. Such assays include, but are not limited to, a measure of the inhibitor to prevent polyvalent interaction. The molecule may, for example, not only slow the rate of infection by blocking attachment to host receptors, but may slow the rate of clearance by blocking the clearance mechanisms. One exemplary assay is phage infection of bacteria. Chu, Y.-H., *et al.*, *Acc. Chem. Res.* 1995, *28*, 461-468.

Table 8 sets forth numerous assays which can used to screen the polyvalent presenters of the present invention for useful properties.

Table 8. Various techniques available to quantitate polyvalent interactions.

Enables measurement of

Technique	System in which this technique has been used successfully	Affinity	Inhibition of Surface-Surface interactions (Affinity plus Steric Stabilization)	Kinetics	Comments
Hemmagglutination Inhibition Assay	1. Inhibitors of the Influenza-Erythrocyte interaction 2. Ab interactions with the surface of wide range of bacteria	no	yes	no	widely used; easily performed; in general, limited to inhibition constants greater than nM
ELISA Assay	Inhibitors of the Influenza-Erythrocyte interaction	yes	no	no	requires synthetic labeling of the polyvalent species; in general limited to dissociation constants greater than 10-50 nM
Fluorescence Activated Cell Corter	Ab interactions with the surface of a cell	yes	yes, in principle	no	requires covalent modification of the Ab; based on the separation of subsequent quantitation of bound and unbound forms
OPTCOL (Optical Collisions using Dual Optical Tweezers)	Inhibitors of the Influenza-Erythrocyte interactions	no	yes	no	enables measurement of physiologically relevent conditions (collision velocity of cells, relative orientation, and other factors are controlled by the user); measurement involves a single cell and a single microsphere coated with viral particles, and the lower limit of measurable inhibition constants is less than 10^{-14} M.
Affinity Capillary Electrophoresis	Dimers of Vancomycin interacting with Dimers of D-Ala-D-Ala	yes	no	no	this technique has much promise, and many extensions to other system are possible and underway
Surface Plasmon Resonance	Ab binding to synthetic surfaces presenting different densities of antigen (DNP, anti-DNP system)	yes	no	yes	requires mg of the polyvalent material (other techniques in this table require 10^2 less in general); other related techniques

Enables measurement of					
Technique	System in which this technique has been used successfully	Affinity	Inhibition of Surface-Surface interactions (Affinity plus Steric Stabilization)	Kinetics	Comments
					include Acoustic Plate Mode and Surface Acoustic Wave, and are all based on the detection of small changes in dielectric constant near an interface
Pipette Suction (Evan Evans)	Cell-Cell	yes, in principle	yes, in principle	no	Based on the quantitation of the energy required to deform complementary cell surfaces during adhesion and separation; may be difficult to perform
Shear Flow (T Springer and others)	1. Neutrophil interaciton with surfaces derivatized non-covalently with different selectins	yes	yes, in principle	no	based on counting large number of events (stuck, non-stuck) under an optical microscope; probability of adhesion is measured as a function of flow rate of solution past surface
Dissociation under influence of gravity		yes	no	no	time to fall off surface is measured, and correlated with strength of adhesion
Optical Microscopy-Counting aggregates (Barry Shur)	Sperm-Egg interactions, and inhibition of those interactions	yes, in principle	yes	no	based on counting a large number of events (bound, unbound) following agitation; probability of adhesion can be measured as a function of concentration of inhibitor; difficult to perform
Atomic Force Microscopy	Surfaces containing streptavidin interacting with surfaces containing biotin	yes	yes, in principle	no	highly sensitive (a single molecule-molecule interaction can be measured); expensive equipment

Enables measurement of					
Technique	System in which this technique has been used successfully	Affinity	Inhibition of Surface-Surface interactions (Affinity plus Steric Stabilization)	Kinetics	Comments
					required; covalent derivization of small objects required
Light Scattering	none	yes	yes, in principle	no	

EX VIVO APPLICATIONS

The present invention also provides for polyvalent presenters for *ex vivo* applications, e.g., materials and systems for passive protection against biological threats, components for systems for decontamination and new diagnostic and characterization systems. In such applications, as described above, the subject polyvalent presenters are designed, ultimately to block infection or intoxication of a subject. In certain embodiments, the subject presenters may additionally comprise one or more antimetabolites. For example passive protection can be provided by the use of protective masks comprising polyvalent presenters that are effective against infectious agents or toxic gases, as well as protective wraps, gowns, dressings and washes for hospitals. Likewise, the polyvalent presenters of the present invention can be incorporated into solutions and processes for decontamination systems.

In another *ex vivo* application the subject polyvalent presenters can be used in, for example, panning assays, or optical assays based on adsorption at surfaces. For such ex vivo applications a wider range of frameworks will be appropriate for use, given that the requirements for in vivo compatibility need not be considered.

MECHANISMS OF ACTION

The mechanisms described below are for discussion purposes and should in no way be construed as limiting to the claimed invention. Furthermore, it should be understood that factors such as "β factor" relate more to objective criteria rather than to mechanism of action. In preferred embodiments the subject polyvalent presenters exhibit enhanced affinity for binding sites B over that seen for monoA. In other preferred

embodiments, the subject polyvalent presenters have a greater specificity than monovalently presented A. By "specificity" it is meant that the nonspecific interaction of polyA with binding sites nonB is reduced over than observed with monoA. In other preferred embodiments, the subject polyvalent presenters produce a biological effect at a lower concentration than that observed for A presented in monovalent form. The polyvalent presenters of the present invention can function by one or more of the mechanisms described below.

For polyvalent interactions as a class, there is no accepted nomenclature. Because there are a number of different ways in which binding sites B (e.g., N receptor sites) can interact with groups A (e.g., N ligands), and because the free energy of binding of an interaction depends strongly on its details, there is probably no simple general nomenclature. As used herein, an interaction between N ligands and N receptors distributed on two entities will be referred to as an Nth-order polyvalent interaction (illustrates a third-order polyvalent interaction). It occurs with free energy of association ΔG_N^{poly}.

The *average* free energy of interaction, ΔG_{avg}^{poly}, between a single ligand moiety and a single receptor moiety in the polyvalent interaction shown is equal to $\Delta G_N^{poly} / N$ (eq. 3). A monovalent receptor-ligand interaction occurs with free energy change ΔG^{mono}; N monovalent, independent receptors interacting with N monovalent, independent ligands occurs with free energy change $N\Delta G^{mono}$. The most useful comparisons of free energies of association involve the same numbers of ligands and receptors, and must be considered thoughtfully if different numbers of ligands and receptors (and different units for equilibrium constants) are involved. Equations 3-5 give the values of the corresponding association constants, K.

$$\Delta G_{avg}^{poly} = \Delta G_N^{poly} / N \qquad \text{(eq 3)}$$

$$\Delta G = RT \ln(K) \qquad \text{(eq 4)}$$

$$K_N^{poly} = (K_{avg}^{poly})^N \qquad \text{(eq 5)}$$

Cooperativity: The magnitude of α

The average free energy of interaction between a ligand moiety and receptor moiety in a polyvalent interaction (ΔG_{avg}^{poly}) can be greater than, equal to, or less than the free energy in the analogous monovalent interaction (ΔG^{mono}) (eqs. 6-8). Following accepted nomenclature in biochemistry, these classes of polyvalent interactions will be referred to as being positively cooperative (synergistic), non-cooperative (additive) or negatively cooperative (interfering), respectively. The convention of defining α as the degree of cooperativity will be followed (Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability;* John Wiley & Sons: New York, 1987). The units of α depend on the order of the polyvalent interaction.

$$\Delta G_{avg}^{poly} = \alpha \Delta G^{mono} \qquad (eq\ 6)$$

$$N\Delta G_{avg}^{poly} = \Delta G_{N}^{poly} = \alpha N \Delta G^{mono} \qquad (eq\ 7)$$

$$K_{N}^{poly} = (K_{avg}^{poly})^{N} = (K^{mono})^{\alpha N} \qquad (eq\ 8a)$$

$$\alpha = \log\left[\frac{\log(K_{N}^{poly})}{\log(K^{mono})^{N}}\right] \qquad (eq\ 8b)$$

Cooperativity in biology has been discussed in the art. (Koshland, D. E.; Neet, K. *Annu. Rev. Biochem* 1968, *37*, 359; Perlmutter-Hayman, B. *Acc. Chem. Res.* 1986, *19*, 90-96). The best-studied positively cooperative systems in biology do not involve polyvalency. For example, the binding of four molecules of oxygen (0_2) to tetrameric hemoglobin occurs with cooperativity; that is, the free energy of binding of the second oxygen to hemoglobin is more favorable than the binding of the first, and $-\Delta G_{avg}^{poly} > -\Delta G_{(first)}^{mono}$. The degree of cooperativity, α in such non-polyvalent systems is greater than one and unitless.

As a class, polyvalent interactions have not been quantitated sufficiently frequently or carefully for positive cooperativity to be inferred unambiguously in even one system. Positive cooperativity for polyvalent systems is part of the present invention.

One example of a positively cooperative polyvalent interaction ($\alpha > 1$) may be the association of pentameric cholera toxin with GM_1, an oligosaccharide portion of GM_1 ganglioside. Cholera toxin consists of five subunits (AB_5). The binding of the toxin to *monomeric* GM_1 (GM1 that has been cleaved from the ceramidyl moieties on the cellular surface) provides a well-studied example of positive cooperativity through purely enthalpically enhanced binding.

The entropy of the first binding event of a monomeric GM_1 derivative to pentameric cholera toxin is equal to the entropy of each subsequent binding event. All differences in the free energies of binding of monomeric GM_1 to the toxin are therefore due to differences in enthalpy. Schoen *et al.* performed calorimetric studies of the binding of pentameric B_5 to five independent GM_1 oligosaccharide units in solution (Schoen, A.; Freire, E. *Biochemistry* 1989, *28*, 5019-24). The binding constant of the first ligand was *less* than the binding constant of the second by a factor of 4. Calculated purely statistically, the binding constant of the first ligand would be expected to be *greater* by a factor of 5/2 than the second binding constant (For an Nth order polyvalent interaction, K_i, the ith binding constant, equals (N+l+i)/i. Therefore for N = 5, $K_2/K_1 = (5/1)/(4/2) = 5/2$). Thus, the binding was enhanced enthalpically.

The binding of pentavalent B_5 to the surface of a cell that is densely covered in GM_1 moieties has been reported to occur essentially irreversibly and more tightly than pentameric cholera toxin to 5 monomeric units of GM_1. Since the enthalpy of interaction is approximately the same. For monomeric GM_1 and for GM_1 immobilized on a surface, this difference in the ΔG of association can be attributed to differences in entropy between these two types of association: the affinity of a polyvalent receptor can be greatly enhanced through multipoint attachment. Because the affinity of the pentamer for the polyvalent surface was not quantitated, the extent of cooperativity, if any, is unknown.

The following are two examples of interactions that are probably negatively cooperative (interfering; $\alpha < 1$); that is, unlike the case of positive cooperativity (hemoglobin and 0_2), the binding of the second ligand to the second receptor occurs with a less favorable free energy than the binding of the first ligand to the first receptor. The first example is the binding of a bivalent antibody (Ab) to ligands found densely packed on a biological surface

(such as a mammalian cell, a virus, or a solid support during an ELISA assay), or immobilized in a polymeric matrix. Tanaka, T.; Suzuno, R.; Nakamura, K.; Kuwahara, A.; Takeo, K. *Electrophoresis* 1986. *7*, 204-209) In general, monovalent binding constants of antibodies for small organic ligands vary significantly, but are in the range 10^5 - 10^8 M-l. For non- or positively cooperative systems, we expect $K_2^{bi} < \left(K^{mono}\right)^2 \sim \left(10^5 - 10^8\right)^2$ M-l. Karush, *et al.*, found that a bivalent Ab for a surface antigen on *Bacillus sp* bound with 30-fold higher affinity than did the corresponding monovalent Ab (a monovalent antibody is one in which the two or more binding sites present in the native protein have been disconnected chemically or enzymatically); that is, $K_2^{bi} = 30K^{mono} < \left(K^{mono}\right)^2 M^{-1}$ (Karulin, A. Y.; Dzantiev, B., B. *Molecular Immunology* **1990**, *27*, 965-971). The binding here is therefore negatively cooperative.

Thus, cooperativity, as defined in the traditional biochemical sense, is neither as *useful* nor as *descriptive* a parameter for polyvalent systems as it is for monovalent ones. Therefore, an empirical metric describing the enhancement of binding of polyvalent systems is needed. By extension from the cooperativity parameter α, we call this new metric β. Thus, although a polyvalent interaction may be qualitatively much stronger than any one of the monomeric interactions contributing to it, these monomeric interactions may still be interfering with (Lees, W. J.; Spaltenstein, A.; Kingery, W. J. E.; Whitesides, G. M. *J. Med Chem.* 1994, *37*, 3419-3433) or indifferent to, one another (the "sticking" of polyvalent ligands to affinity gels during affinity chromatography probably represents such a case). Only by a quantitative comparison of polyvalent and monovalent interactions is it possible to establish the nature of cooperativity.

Enhanced affinity in polyvalent interactions: the magnitude of β

In many polyvalent systems, the number of ligand-receptor interactions, N, is unknown. For example, polyvalent inhibitors of agglutination of erythrocytes by influenza virus, composed of a polyacrylamide backbone, with a certain fraction of the side chains terminated in sialic acids (SA) have been shown to interact specifically with multiple hemagglutinin (HA) receptor sites on the surface of influenza virus. These molecules prevent the interaction of influenza with its target cell. Using a polymer whose side chains present both SA groups and a small number of biotin groups (as a ligand for attachment of

enzyme-conjugated streptavidin in a subsequent step in the assay), the binding of this polymeric polyvalent inhibitor to the surface of the virus using an ELISA-like assay can be measured. The surface-bound virus was incubated with varying concentrations of polymer containing SA, and the amount of bound polymer was measured indirectly through an enzyme-linked streptavidin-biotin interaction. The measurable quantities in constructing a binding isotherm were the concentration of SA, and the amount of SA contained on polymer bound to the surface of the virus. Neither the number of polymers bound to the virus nor the number (N) of SA groups on each polymer that were bound to HA receptor sites were known precisely. This interaction between polymer and virus can be analyzed by determining the amount of bound polymer as a function of [SA]. At half-maximal binding, $1/K^{ELISA} \equiv$ [SA], where K^{ELISA} is equivalent to an association constant (eq 9). Since the value of N in this polyvalent interaction is unknown here, no statement regarding cooperativity (α) is possible. The value of K^{mono} in this example is $5x10^2$ M^{-1}; the value of K^{ELISA} for our best inhibitor is 10^8 M^{-1}.[20] That is, monomeric SA binds half-maximally at [SA] = $2x10^{-3}$ M, whereas the polymer containing SA binds half-maximally to the surface of the virus at [SA] = 10^{-8} M. Thus, the polyvalent inhibitor may therefore be useful regardless of its value of α.

An enhancement factor β, defined as the ratio of the two association constants

$$K^{ELISA} = \beta K^{mono} \qquad \text{(eq 9)}$$

is, thus, preferable to cooperativity (α) in discussion of enhanced affinity of polyvalent systems: molecules that have high values of β are useful, regardless of whether the interactions that generate them are cooperative or not. In any system where N is unknown, but the total quantity of bound polyvalent molecule is known, β will be a useful parameter. Only if the value of N is known can the precise relationship known between ΔG_N^{poly}, K_N^{poly} and β be calculated (eqs 10, 11).

$$\Delta G_N^{poly} = \Delta G^{mono} - RT\ln(N\beta) \qquad \text{(eq 10)}$$

$$\beta = (K_N^{poly}/N)/K^{mono} \qquad \text{(eq 11)}$$

A polyvalent presenter of the invention where group A is a saccharide usually has a β value that is greater than or equal to about 10^8, preferably. A polyvalent presenter of the invention where group A is a non-saccharide, particularly where group A is a non-natural ligand, usually has a β values that is greater than or equal to about 10, preferably, greater than about 10^2, more preferably, greater than about 10^4. Non-natural ligands generally are not polyvalent although the receptor to which it binds may have multi-valency.

Enthalpy of a Polyvalent Interaction

The ΔG_N^{poly} is comprised of enthalpic (ΔH_N^{poly}) and entropic (ΔS_N^{poly}) components (eq 12). As a first approximation, the value of ΔH_N^{poly} is the sum of the enthalpies of N monovalent interactions, $N\Delta H^{mono}$. This value may be made either larger or smaller by other interactions around the active site.

$$\Delta G_N^{poly} = \Delta H_N^{poly} - T\Delta S_N^{poly} \qquad \text{(eq 12)}$$

Enthalpically Enhanced Binding.

In some circumstances, the binding of one ligand to a receptor with a given enthalpy may cause the next ligand to bind to its receptor with greater enthalpy; that is, the value of ΔH_{avg}^{poly} is in this case more negative (more favorable) than the value of ΔH^{mono}. Such binding is *enthalpically enhanced.*

Enthalpically Diminished Binding.

If the binding of one ligand to its receptor *interferes* with the next binding event, the enthalpy of the polyvalent interaction is less favorable than that expected for N equivalent monovalent interactions. Such binding is *enthalpically diminished.* Enthalpically diminished binding can occur when formation of multiple ligand-receptor interactions between two polyvalent entities requires energetically unfavorable molecular conformations. As a rule of thumb, the more conformationally rigid the polyvalent entity, the more likely that

spatial mismatches between ligand and its receptor will result in enthalpically diminished binding (unless the geometric fit between ligand and receptor is exact).

Entropy of interaction

Understanding the entropy of polyvalent interactions is essential in understanding the relationship of monovalent to polyvalent binding. Incomplete understanding of entropy in the design of polyvalent inhibitors has resulted in many synthetic polyvalent molecules that are only marginally more effective than their monovalent counterparts.

The total entropy of a polyvalent interaction ΔS_N^{poly} should be considered in terms of contributions from changes in translational ($\Delta S_{trans,N}^{poly}$), rotational ($\Delta S_{rot,N}^{poly}$) and conformational ($\Delta S_{conf,N}^{poly}$) entropies of the receptors and ligands on association, and a contribution accounting for changes in the entropy of the surrounding water ($\Delta S_{H2O,N}^{poly}$); often largely due to the entropy of hydrophobic interactions (eq 12).

$$\Delta S_N^{poly} = \Delta S_{trans,N}^{poly} + \Delta S_{rot,N}^{poly} + \Delta S_{conf,N}^{poly} + \Delta S_{H2O,N}^{poly} \qquad \text{(eq 12)}$$

Translational and Rotational Entropies.

The translational entropy of a molecule, ΔS_{trans}, arises from its freedom to translate independently through space; the value of ΔS_{trans} is related to the logarithm of its mass, M ($\Delta S_{trans} \propto 1n(M)$), and inversely to the logarithm of its concentration ($\Delta S_{trans} \propto 1n[L]^{-1}$). The rotational entropy, ΔS_{rot}, arises from the freedom of the particle to rotate around all three of its principle axes, and is related logarithmically to the product of its three principle moments of inertia, I_χ, I_γ and I_Z ($\Delta S_{rot} \propto 1n(I_X I_Y I_Z)$). The values of ΔS_{trans} and ΔS_{rot} for a particle are, therefore, only weakly (logarithmically) dependent on its mass and dimensions. To a first approximation, the translational and rotational entropies of *all* particles -- receptors, ligands, receptor-ligand aggregates -- are equal. When two particles associate, a total of three translational and three rotational degrees of freedom are lost. If the differences in the masses of the particles are ignored (often, the masses of particles are

within a factor of 10; almost always, they are within a factor of 100), then the total translational and rotational entropic cost of associating the two particles, whether they be monovalent or polyvalent, is approximately the same provided they are at the same concentration. In biology, concentrations of molecules can vary over more than nine orders of magnitude (mM to pM). Even though the translational entropy is only logarithmically dependent on concentration, such wide range makes knowledge of the concentration of interacting particles essential to estimating the importance of translational entropy: this cost increases with decreasing concentration.

Conformational Entropy.

Entropy and enthalpy can have partly compensating effects on the affinity of polyvalent interactions: where conformational flexibility increases the conformational entropic cost of association, the same flexibility increases the likelihood that all ligand-receptor interactions can occur without energetic strain.

This loss in conformational entropy on association of a polyvalent ligand with a polyvalent receptor has been notoriously difficult to quantitate. The change in entropy on freezing a single, rotating carbon-carbon bond is approximately 0.5 kcal/mol (ref). The range of values for other single bonds is 0.1 - 1.0 kcal/mol. The maximum loss in conformational entropy would occur if all bonds that were initially rotating freely lost all degrees of torsional freedom on complexation. The upper estimate for this loss in ΔS_{conf} is then approximately 0.5N kcal/mol, where N is the number of single bonds in the tether linking two ligands or receptors. For a long flexible chain, this number can be large: for a triethyleneglycol spacer, it can be unfavorable to the extent of as much as 10 kcal/mol.

Entropy of Solvation.

A final contribution to the total entropy of association in aqueous system is the change in the entropy of the surrounding molecules of water, ΔS_{H2O}. The major contributor to interactions in water (both hydrophobic and interactions involving polar groups) is the release of organized water from exposed faces of the biological molecules, and the resulting increase in entropy. Quantitative measurements and predictions of hydrophobic interactions have been reviewed extensively (Blokzijl, W.; Engberts, B. F. N. *Angew. Chem. Int. Ed Engl.* 1993,*32*, 1545-1579). These terms will usually be similar per

ligand, in monovalent and polyvalent systems (unless the linker changes conformation or associates with the surface of the receptor, and as a result, changes its association with solvent on complexation).

There are a number of classes of polyvalent interaction whose entropic characteristics differ markedly. Interactions can occur when both species are initially freely diffusing in solution (the initial six degrees of translational freedom for two such particles are reduced to three degrees following complexation), and we classify these interactions as three-dimensional (3D); when one or both species are restricted to diffusion in a plane (classified as two-dimensional 2D); and when the interacting species are restricted to lines (classified as one-dimensional 1D). The translational and rotational entropic costs of association depend on the number of translational and rotational degrees of freedom lost on complexation. The cost (the combined values of ΔS_{trans} and ΔS_{rot}) is greatest for particles associating in 3D, less in 2D, and least in 1D. That is, the entropic cost of association between N ligands freely diffusing in 2D (such as ligands tethered to the surface of a cell) with N receptors also freely diffusing in 2D (such as trans-membrane proteins on the surface of another cell) is less than for the interaction of these species diffusing independently in 3D.

Kinetics and Enhanced Affinity

In certain embodiments the subject polyvalent presenters function by having extremely low off-rates, such that they are effectively "permanently" bound with regard to the time frame of a biological event relevant to therapy. This difference in kinetics between polyA and monoA, i.e., the lower off-rate for the polyvalent form is another mechanism by which the subject presenters are different from monoA.

Studies of the kinetics for high affinity interactions suggest that the enhancement is mostly due to decreases in the rate of dissociation (k_{off}) of the two polyvalent entities rather than to increases in the rate of association. Binding of anti-DNP Ab to DNP-lys, relative to the binding of the same Ab to the DNP-covered surface of ΦX174, established that the values of k_{on} for binding to the surface differed by only a factor of 2 (k_{on} (surface) ~ 3.7×10^7 $M^{-1}s^{-1}$, k_{on} (DNP-lys) ~ 8×10^7 $M^{-1}s^{-1}$), where the values of k_{off} differed by a factor of 10^4 (k_{off} (surface) ~ 3.3×10^{-4} $M^{-1}s^{-1}$, k_{off} (DNP-lys) ~ $1.0s^{-1}$). Since the *rate* of a process is related qualitatively (and, very often quantitatively) to

its *thermodynamics*, (Agmon, N.; Levine, R. D. *Chem. Phvs. Lett.* 1977, *52*, 197-201) then these measurements are intuitively consistent with polyvalency: the thermodynamic cost of the first ligand-receptor interaction between two polyvalent entities is approximately the same as the thermodynamic cost of the analogous monovalent interaction; it is therefore plausible that the rates of association might be similar. Dissociation of species interacting polyvalently requires breaking N ligand-receptor interactions; it is therefore plausible that dissociation occurs more slowly in the polyvalent interaction than in the corresponding monovalent one.

Steric Inhibition

In certain embodiments the polyvalent presenters of the present invention function by "steric inhibition". Steric inhibition is a new strategy in the design of effective pharmaceutical agents. In the case of infectious agents, for example, polyvalent inhibitors of attachment may be designed that involve any molecule that binds tightly to the surface of the infectious agent, i.e., the polymers that present molecules need not be directly involved in attachment. For example, it is possible to build polymers that prevent the adhesion of influenza virus to erythrocytes by presenting groups that bind to the neuraminidase (NA) on the surface of the virus. Choi, S.-K.; *et al. Chem. & Biol.* 1996,3, 97-104 The NA site is commonly regarded by those of skill in the art as not mediating adhesion, thus the anti-adhesive effect observed with a polymer directed toward it may occur as a result of the attachment of the polymeric gel layer to the viral surface. This effect may be "pure" steric inhibition, i.e., with no entropically enhanced occupancy of the active site of hemagglutinin, the protein that the virus normally uses in adhesion. Thus, polyvalent presentation of a drug may change the original mechanism of action for that drug. There are currently no known examples of such drugs.

The mechanism of steric inhibition is believed to be more related to colloidal stabilization than to receptor-mediated events, although it does depend on receptor-directed specificity to target the polymer to the appropriate binding sites B. Bringing two moieties or groups together when one or both is coated by a gel layer is unfavorable both entropically (because the conformational mobility of the water-swollen polymer is decreased on approach to another surface) and enthalpically (because of unfavorable osmotic effects). Polymeric presenters are unique in that they function by this type of mechanism, although there are

other, related mechanisms that may appear with liposomes (Kingery-Wood, *et al. J. Am. Chem. Soc.* 1992,114, 7303-7305) and dendrimers. (Roy, R.; Tropper, F. D. *J. Chem. Soc., Chem. Commun.* 1988, 1058-1060; Roy, R. *et al.* American Chemical Society: Washington, D. C., 1994).

Adsorption and Precipitation

In certain embodiments the subject polyvalent presenters function by mediating the adsorption of binding sites B on a surface. By such a mechanism, binding sites B, e.g., a viral particle, may be effectively removed from solution and cleared by a subject. In other embodiments, the polyvalent presenters function by being involved in precipitation or aggregation.

PHARMACODYNAMICS

The framework that is chosen and its inherent properties will influence the pharmacodynamics of the polyvalent presenter. For example, two of the properties that are considered when designing polyvalent presenters of the present invention are solubility and size.

Solubility

In most embodiments the polyvalent presenters of the present invention will be more water soluble than conventional pharmaceutical agents. (e.g., in the mg/ml range or higher). Solubility (and size as described *infra*) can influence the pharmacodynamics of the polyvalent presenter. For example, solubility of the presenters may influence one or more of the related characteristics described below.

For example, solubility can influence the clearance profile of the subject polyvalent presenters. Clearance can be dramatically increased as the solubility of a molecule increases. The kidney tends to filter water soluble molecules more rapidly. Also, the rate of drug clearance is directly proportional to the frequency of drug administration.

The water solubility of the subject presenters can also influence the duration of action of the presenters. In preferred embodiments, the subject presenters have a longer duration of action than does monovalently presented A.

In other embodiments the solubility of polyA can influence therapeutic index. As used herein, the term "therapeutic index" refers to the (LD50/ED50) as can be determined by methods well known in the art. The therapeutic index as used herein is meant to be calculated on a per-group A basis. The therapeutic index is inversely proportional to the frequency of drug administration. Owing to the lower clearance rates of the subject presenters, polyA will be able to be administered to a subject in at less frequent doses than monoA. Moreover, polyA will exhibit a lower concentration variance over time in a subject at the site of interest than monoA, since the rate of clearance of these drugs can be very slow, they can stay at a more even concentration in the plasma, i.e., polyvalent drugs will have a reduced difference between maximum and minimum concentration at the site of interest (e.g., lower trough-peak variance). Since polyvalent molecules are large they have the distinct advantage that their lifetimes can be significantly longer than those observed for small molecules. Owing to the lower clearance rates of the subject presenters, polyA will be able to be administered to a subject in at less frequent doses than monoA. These longer half-lives are advantageous for a variety of reasons. For example: (i) patient compliance and patient happiness would increase as frequency of drug administration is decreased; (ii) patients can be discharged earlier from hospitals than is currently possible; (iii) drugs can be administered that have lower therapeutic indices than is currently possible.

The solubility of the subject polyvalent presenters can also influence compartmentalization of polyvalent presenters. Other factors besides solubility also may be responsible for the compartmentalization and all forms of compartmentalization, e.g., inclusion or exclusion, are intended to be part of this invention. For example, the distribution coefficient between water and an organic solvent (mimicking the biological environment) is important. Polymeric, polyvalent species having high molecular weight will, in general, not cross biological membranes effectively. This characteristic can, in certain embodiments, make it preferable that they be administered by direct delivery into the compartment of interest. Alternatively, this property means that the polyvalent presenters can be excluded from the undesirable compartments. As examples, intravenous injection accesses the vascular compartment; intrathecal injection accesses the cerebrospinal fluid and the central nervous system; the oral route accesses the gastrointestinal tract; eye drops access the ocular compartment, creams and ointments access the epithelium; catherization accesses the biliary tree and the pancreas and the gall bladder, as well as the cystourethral system, and the

vagino-tuboovarian system; and finally inhalation accesses the bronchioalveolar compartment.

Molecules may also be designed to keep them out of particular compartments. Examples of areas where this concept may be useful include obstetrics (keeping agents out of the fetal circulation), and agents that are toxic to the kidney (keep agents from being taken up by the kidneys by keeping them in circulation), or to keep presenters out of the central nervous system.

Polyvalent presenters can also have a tendency to stay localized at a site of interest, with the advantage of reducing systemic toxicity and maximizing local concentrations.

In addition, molecules can be designed that are not confined to a compartment when that property is desirable. For example, in the acute setting, it may be undesirable to have a long-acting agent, but it may be important to have the increased potency that a polyvalent agent can bring. In these applications, polymers may be designed to be of intermediate size, or may comprise cleavable connectors. These molecules will be potent but sufficiently small to be filtered by the kidney and thereby cleared.

Some examples of specific compartments include: the eye (e.g., agonists and antagonists of tearing during surgical procedures or antibiotics), the GI tract (e.g., agonists and antagonists of peristalsis (cholinergic agonists) (cathartic) agonists and antagonists of muscle tone (glucagon) (prior to double contrast barium studies). Other examples include: the CNS, the urogenital system (e.g., kidney, ureter, or bladder, vagina, uterus, fallopian tubes (e.g., contraceptives), and the bronchial tree (e.g., antiasthmatic medication, or cystic fibrosis therapies). Still other examples of compartments include surface (e.g., skin and mucus membranes for topical applications); the ear canal and middle ear (e.g., antibiotics, antivirals), the blood (e.g., intravenous injection, as well as transdermal and transmucosal delivery vehicles). Other examples of compartments include intravasular, extravasular, cerebrospinal fluid, the bronchoalveolar space, the pleural space, the peritoneal space, the ocular compartment, the topical compartment, the urinary tract, and the reproductive tract, e.g., vaginal, uteral, and fallopian tubes.

Size

The size of the polyvalent presenter can influence the same properties of duration of action, therapeutic index, compartmentalization, and clearance profile as can solubility (as described *supra*).

The term "size" is intended to encompass both to molecular size in terms of carbohydrates (i.e., Stokes radius) and molecular weight (kD) in terms of proteins. Polyvalent presenters of molecular size above 60 kD or greater than 50Å mean hydrodynamic diameter are more likely to be compartmentalized than are molecules of smaller molecular size. In particularly preferred embodiments polyvalent presenters of the present invention are greater than 2kD and /or 5nM 50Å mean hydrodynamic diameter.

The size of polyvalent presenter selected may vary with the "state" to be treated. For example, for parenteral applications, low molecular weight compounds (less than about 10,000 MW) will generally be cleared more rapidly. Alternatively, larger compounds that contain cleavable linkages that link units small enough to be cleared when released can be used. Molecules of size greater than that of a 60-70 kDa protein may not be filtered effectively by the kidney, which is important in instances where the polyvalent presenters are to be used in the bloodstream. When used for oral, lung or topical applications, the materials may not need to be cleared or degradable *in vivo*.

For drugs that have essentially a zero clearance because they are too large to be cleared by the kidney, and which are not taken up and cleared by the liver, clearance, when desirable, can be induced by a number of mechanisms. For example, small molecular weight pieces (of a size can easily be cleared by the kidney) can be joined by connectors that hydrolyze at a significant rate in the serum. Alternatively, such low molecular weight pieces can be joined by connectors that are hydrolyzed by agents (e.g., enzymes) naturally present in the plasma. In another embodiment, such small molecular weight pieces can be joined by connectors that can be cleaved by agents (e.g., a second drug, either polyvalent or monovalent) that is taken at the time that clearance of the polyvalent presenter is desirable. An example of such a second agent may be a thiol or a chelating agent, and examples of linkages susceptible to these agents may be disulfides and organometallic links).

In general, high molecular weight species cannot access the blood by oral administration. High molecular weight systems are, nonetheless, useful for other application. In certain embodiments they will be made available to the subject by transmembrane

permeation (across nasal or pulmonary membranes following administration as aerosols). Some polyvalent species may be taken up through cells in the gut, e.g., by formulation to survive the digestive process or administration as suppositories. Large polyvalent agents, because they will not pass from the lung, gut, or respiratory passages into the systemic circulation, can be advantageous in that they have limited side effects.

ADDITIONAL EMBODIMENTS

In certain embodiments it will be desirable to treat a subject with one or more polyvalent presenters and, in addition, with a monovalent inhibitor. Such monovalent inhibitors may or may not interact with the same binding site B as does group A on the polyvalent presenter. For example, monovalent inhibitors of influenza neuraminidase (NA), a hydrolytic enzyme present on the surface of influenza virus, enhance the ability of polyacrylamide presenting HA inhibitors to prevent hemagglutination (Choi, S.-K.; Mammen, M.; Whitesides, G. M. *Chem. & Bio.* **1996**, 3, 97-104). NA sites on the surface of virus act as secondary binding sites for SA. Adding monovalent inhibitors of NA prevents the secondary binding of SA leading to an increase in the effectiveness of these polymeric inhibitors, probably due to increased steric stabilization. In still other embodiments the subject presenters can be used in conjunction with any other method of treatment.

The time to onset of action for a polyvalent presenter also may be important. Multivalent hapten-carrier conjugates (which in some embodiments are not intended to be part of this invention) typically require a long period of time to elicit a useful biological response. The polyvalent presenters of the present invention can elicit a useful biological response or a therapeutic effect within a day, or preferably within twelve hours, within five hours, within one hour or in some situations a substantially immediate response or effect, e.g., within several minutes down to a second, e.g., when asthma is being treated.

The following invention is further illustrated by the following examples, which should not be construed as further limiting. The contents of all references, provided manuscripts (see Appendices A and B), pending patent applications and published patents, cited throughout this application (including the "Background" Section) are hereby expressly incorporated by reference.

EXAMPLES

Example 1. The Preparation of Polyvalent Presenters for Facilitating the Treatment of Influenza

Section A-The Generation and *In Situ* Evaluation of Libraries of Derivatives of Poly(Acrylic Acid) Presenting Sialosides as Polyvalent Inhibitors of Influenza-Mediated Hemagglutination

A simple, micro-scale method for generating and evaluating libraries of derivatives of poly(acrylic acid) (pAA) that present mixtures of side chains that influence their biological activity was developed. Using this method, derivatives of pAA having *N*-acetylneuraminic acid (NeuAc-L-NH_2) as a side chain, pAA(NeuAc-L), were generated and assayed for ability to inhibit hemagglutination (HAI) of chicken erythrocytes by influenza virus A (X-31); the constant (K_i^{HAI}) describing this inhibition is calculated on the basis of total NeuAc groups in solution. Using combinations of NeuAc-L-NH_2 and one of 26 different primary amines RNH_2, a variety of ter-polymeric pAA(NeuAc-L; R) ($\chi^{NeuAc\text{-}L} \sim 0.05$; $\chi^{R} \sim 0.06$) were generated and assayed. These polymers (pAA(NeuAc-L; R)) belong to a new class of polymeric, polyvalent sialosides that are potent inhibitors of the adsorption of influenza virus to erythrocytes.

The method is based on the conversion of poly(acrylic acid anhydride) (pAAn) to derivatives of pAA by reaction with various amines RNH_2, in water. These derivatized polymers were prepared by ultrasonication of the reactants directly in the wells of a microtiter plate; then assayed in the same plate with no further manipulation.

Influenza initiates infection by adsorption to the surface of mammalian cells through the multiple interactions of the viral protein hemagglutinin (HA) and clusters of NeuAc moieties expressed on the cellular surface. Monomeric sialosides are, in general, weak inhibitors of the adsorption of virus (as measured by inhibition of hemagglutination) (see, e.g., Sauter, N. K. *et al.*, Biochemistry **1989**, *28*, 8388).

pAA was synthesized having multiple R groups as side chains, pAA(R) by sonicating a suspension (0.12 mg/μL) of poly(acrylic acid anhydride) (pAAn) (see, e.g.,

Jones, J. F. *J. Polymer Sci.* **1958**, *33*, 15.; Brotherton, T. K.; Smith, Jr, J.; Lynn, J. W. *J. Org. Chem.* **1961**, 26, 1283) and an aqueous solution of an amine RNH_2 (0.1 M) contained in a 250μL well of a microtiter plate (eq 1 See Appendix A). Solutions of co-polymers pAA(NeuAc-L) were prepared by reacting of NeuAc-L-NH_2 (**1**, **2**, **3** or **4**) with poly(acrylic acid anhydride) (pAAn) using different numbers of molar equivalents (mol eq.) of NeuAc-L-NH_2 to pAAn (eq 1). The polymer for which mol eq. = 0 is homo-polymeric pAA obtained from the sonication (hydrolysis) of pAAn alone in PBS buffer (137 mM NaCl, 2.7 mM KCl, 7.7 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.05% NaN_3), pH 12. Co-polymeric pAA(NeuAc-L) for which mol eq. > 0 was generated in microtiter plates with 96 conical-bottom wells as follows: (i) placing 6 mg of pAAn into a well; (ii) soaking the powder with a variable amount (19 - 100μL) of 0.1 M NeuAc-L-NH_2 in PBS buffer, pH 12; (iii) immediately sealing the plate (taping four sides of a plate with parafilm® and placing a cover tightly), and then ultrasonicating (Fisher ultrasonic bath-type cleaner) the mixture for 0.5 h: the sonication also increased the temperature of water in the bath (and the reactants) slowly up to ~50°C. Each solution of generated co-polymers (pH ~3) in a well was neutralized to pH ~7 by adding 60 μL of 1.0 M NaOH, and adjusted to 100 or 200 μL (total volume) with PBS, pH 7 before the HAI assay. The above protocol was easily extended to the preparation of ter-polymers pAA(NeuAc-L; R); here, a three-component mixture (pAAn (6 mg), 50 μL of 0.1 M NeuAc-L-NH_2 (**1** or **3**) and 30μL of 0.2 M RNH_2) was sonicated.

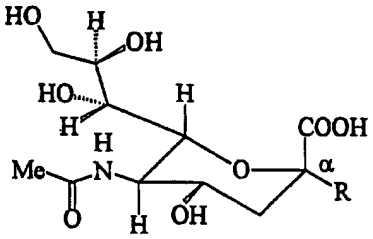
The resulting pAA(R) was characterized by examining ^{1}H-NMR (D_2O) spectra of lyophilized reaction mixtures (both as a crude mixture and following dialysis) and by gel permeation chromatography (M_W = 39.5 kDa, polydispersity = 1.91; polysaccharide standards). By comparing the integrated intensity of NMR signals from free RNH_2 before and after sonication, the yields of incorporation of RNH_2 were observed. The ^{1}HNMR signals of R from pAA(R) were distinguished readily from those of free unreacted RNH_2 by their shape (the lines due to polymer-attached species are relatively broad) and by their chemical shift (the δ valure of CH_2 or CH groups next to the amide group are shifted downfield). The percent yield of incorporation of RNH_2 as an amide group is on the basis of RNH_2:

$$\text{yield of incorporation (\%)} = \frac{\text{number of moles of –(CONHR)}}{} \times 100(\%)$$

(number of moles of used RNH_2)

the average value was ~90% (±5) from experiments using five different amines RNH_2 (4-aminobenzoic acid, 6-aminohexanoic acid, *N*-methylhydroxylamine, (L)-arginine and **1** (NeuAc-L_1-NH_2)). Because amide formation and hydrolysis of anhydride groups were occurring competitively, the efficiency of the former process was affected by the relative reactivity of each RNH_2, and was also sensitive to both the pH of the aqueous solutions of RNH_2 (optimal pH ~ 7 and 12 for aromatic and aliphatic amines, respectively) and to the number of molar equivalents of RNH_2 to pAAn (optimal mol eq. < 0.2).

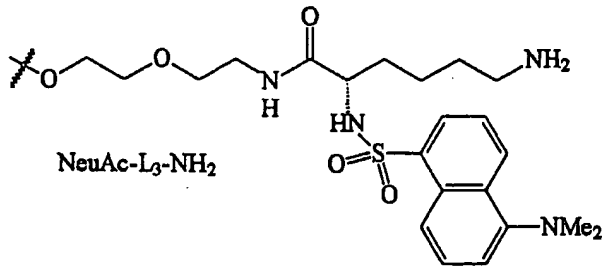
Co-polymeric derivatives of pAA were generated presenting NeuAc-L as a side chain (pAA(1) - pAA(4)) by using this quasi-solid-phase synthetic method with derivatives of NeuAc having different linking groups L (**1** - **4**; NeuAc-L_n-NH_2; see Figure 1 or Scheme 1) (for synthesis of NeuAc-L-NH_2 **1** and **2**, see Sparks, M. A.; Williams, K. W.; Whitesides, G. M. *J. Med. Chem.* **1993**, *36*, 778; Ogura, H. *et al.*, *Carbohydr. Res.* **1986**,*158*, 37; Lees, W. J. *et al. J. Am. Chem. Soc.* **1994**, *37*, 3419. NeuAc-L-NH_2 (**3**, **4**), were prepared as described below. **3** and **4** were used for incorporation into side chains of the polymer, because aromatic moieties in the middle of the linkage may enhance the binding affinity of monomeric NeuAc-L to HA site: Watowich, S. J.; Skehel, J. J.; Wiley, D. C. *Structure* **1994**, *2*, 719).



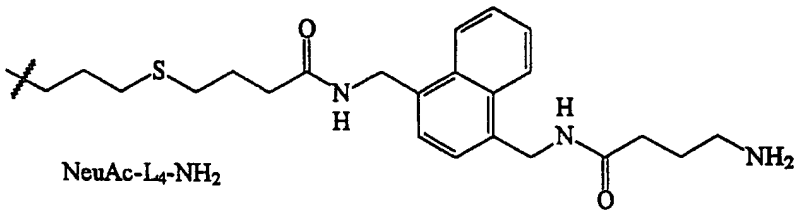
NeuAc R = OH; N-Acetylneuraminic Acid or Sialic Acid

1 R = $(CH_2)_3S(CH_2)_2NH_2$; NeuAc-L_1-NH_2

2 R = $O(CH_2)_2O(CH_2)_2NH_2$; NeuAc-L_2-NH_2

3 R = 

NeuAc-L_3-NH_2

4 R = 

NeuAc-L_4-NH_2

Scheme 1

Following sonication, the crude solutions of polymers were evaluated immediately for hemagglutination inhibition (HAI) activities using an assay based on chicken erythrocytes and influenza virus A (X-3 1) (see, e.g., Choi, S.-K.; Mammen, M.; Whitesides, G. M. *Chem. & Biol.* 1996, *3*, 97; Lees, W. J. *et al. J. Med. Chem.* 1995, *37*, 3419; and references cited therein).

Table 9 below gives the values of K_i^{HAI} (the lowest concentration of NeuAc-L groups from pAA(NeuAc-L) in solution that prevents hemagglutination) at various molar equivalents of NeuAc-L-NH_2. The molar equivalent of NeuAc-L-NH_2 is related directly to the mole fraction of NeuAc-containing side chains in the polymer, $\chi^{NeuAc\text{-}L}$. Table 9 also shows three other derivatives of pAA (pAA(2) - pAA(4)) with HAI activities in the

(sub)micromolar range. In comparison, the HAI activities of all monomeric sialic acids (**1** - **4**) were low, $K_i^{HAI} \geq 5$ mM.

The hemagglutination inhibition activities of pAA(NeuAc-L) and libraries of pAA(NeuAc-L; R). Libraries of pAA (NeuAc-L; R) are shown in Table 9.

Table 9. The hemagglutination inhibition activities of pAA(NeuAc-L) and libraries of pAA(NeuAc-L; R).

Polymer	mol. eq. RNH_2	mol. eq. NeuAc-L-NH_2	$K_i^{HAI}{}_{(\mu M)}$[a]
pAA(1)		0	15000[b]
		0.04(1)	27
		0.06(1)	13
		0.08(1)	3.9
		0.10(1)	3.4
		0.11(1)	4.4
		0.12(1)	1.1
		0.14(1)	1.1
		0.17(1)	0.50
		0.21(1)	0.30
pAA(2)		0.11(2)	0.80
pAA(3)		0.11(3)	0.20
pAA(4)		0.11(4)	3.1
pAA(1;R)	0.12(RNH_2)	0.10(1)	

Table 9 (continued)

	RNH_2	
	3-aminobenzoic acid	1.5
	3-amino-5-hydroxybenzoic acid	3.1
	4-aminobenzoic acid	3.1
	4-amino-2-hydroxybenzoic acid	3.1
	4-aminobenzenesulfonic acid	1.5
	2-aminonicotinic acid	1.5
	N-methylhydroxylamine	1.5
	(D)-2-amino-2-deoxyglucose	2.2
	(D)-2-amino-2-deoxymannose	0.055
	1-amino-1-cyclopropanecarboxylic acid	1.1
	1-amino-1-cyclopentanecarboxylic acid	0.20
	1-amino-1-cyclohexanecarboxylic acid	0.028
	aminocyclohexane	0.0043
	(L)-arginine	1.5
	(L)-glutamate	2.5
	(L)-histidine	1.5
	(D)-4-hydroxyproline	1.5
	(DL)-leucine	0.30
	(L)-phenylalanine	0.024
	(L)-4'-nitrophenylalanine	0.048
	(L)-phenylalanine methyl ester	0.024
	1-amino-2-phenylethane	0.0021
	(L)-3-(2'-naphthyl)alanine	0.00050
	(L)-tryptophan	0.0043
	(L)-Gly-(L)-Gly-(L)-Gly	3.1
	(L)-Gly-(L)-Phe	1.5
pAA(3;R)	0.13 (RNH_2) 0.11(3)	
	RNH_2	
	1-amino-2-phenylethane	0.0015
	(L)-3(2'-naphthyl)alanine	0.00070

Section B-Polyvalent Presenters Having Two Types of "A" Groups

The method of Section A was extended (eq 1) to generate libraries of ter-
5 polymers, pAA(NeuAc-L; R) which polyvalently present both NeuAc-L and one other R group, by sonicating a three-component mixture of NeuAcL-NH_2, RNH_2 and pAAn.

Table 9 (above) summarizes the values of K_i^{HAI} of pAA(NeuAc-L; R), obtained from combination of NeuAc-L-NH_2 (mol eq. = 0.10) and one of 26 different RNH_2 (mol eq. = 0.12). Several pAA(1; R) (and pAA(3; R)) showed activities enhanced by factors

of 100 to ~7000 relative to the parent co-polymeric pAA(1) (mol. eq. of 1 = 0.10) in which there is no R group (note that the HAI assay requires the use of a finite amount of virus, and can not measure accurately the effectiveness of inhibitors with $K_i^{HAI} < 1$ nM). Although we measured the activities directly from crude pAA(NeuAc-L; R), several control experiments confirmed that these ter-polymers (and not pAA(NeuAc-L)) were responsible for the high activities. Typically, the incorporation of derivatives of hydrophobic or aromatic amino acids enhanced the activities greatly. We have now demonstrated that certain structural features (and not hydrophobicity alone) are particularly effective in decreasing the value of K_i^{HAI}. We conclude that certain non-sialoside groups, although they showed no HAI activity by themselves, enhanced the activities of pAA(NeuAc-L; R) by factors up to ~10^4.

The best of these pAA(NeuAc-L; R) belongs to a new class of hemagglutination inhibitors that have unusually high activities at relatively modest mole fractions of NeuAc-L (~5%) and R (~6%): each 1% in mole fraction of NeuAc-L or R is equivalent to ~ 6 side chains (per polymer molecule). This finding emphasizes the importance of combinations of side chains in modulating the activities of these polyvalent presenters.

The methods described in Sections A & B streamline the generation of derivatives of pAA and the evaluation of the biological activities of these polymers by carrying out both synthesis and assay in the wells of microtiter plates. The methods allow convenient screening of libraries of polymers presenting multiple combinations of side chains at controlled mole fractions. Since microtiter plates assays are routine in biology and medical sciences, this method can serve generally for screening and obtaining leads for a range of agglutination interactions and other processes that might be influenced by polyvalent inhibitors. This procedure is a rapid, economical method for synthesizing and screening polyvalent, polymeric inhibitors for bioactivity.

Example 2-The Preparation of A Polyvalent Presenter for Facilitating the Inhibition of Platelet Aggregation

RGD (L-Arg-L-Gly-L-Asp) is a recognition sequence for the binding of fibrinogen to platelets. Analogs of RGD inhibit the interaction between fibrinogen and the platelet membrane glycoprotein IIb/IIIa complex, thus acting as platelet aggregation inhibitors (see Phillips, D. R., *and Cell*, 65:359 (1991)).

A polyvalent presenter that presents RGD as a ligand is prepared as follows:

RGD immobilized on a solid support at the C-terminus is prepared according to known methods of solid-phase peptide synthesis (SPPS), e.g., on Wang resin. Solid-supported RGD is reacted with succinic anhydride to provide immobilized succinylated RGD, which is then coupled with mono-BOC-protected 1,6-diaminohexane (e.g., with DCC/HOBt). Cleavage of the derivatized RGD from the resin and concomitant removal of the BOC group is achieved by acid treatment, followed by purification (e.g., by HPLC) to provide RGD functionalized with a linker moiety having a terminal amino group.

Polysuccinimide (prepared according to known methods; see, e.g., U.S. Patent No. 5,484,878 for preparation of polysuccinimide from aspartic acid) is suspended in a solvent such as dimethylformamide (DMF) or dimethylacetamide, and the linker-functionalized RGD is added to the suspension with stirring. After the aminolysis of the polysuccinimide is complete, the reaction mixture is diluted with aqueous base to hydrolyze the unaminolysed polysuccinimide, and then dialyzed to remove impurities, providing a poly(aspartic acid) backbone functionalized with a linker bearing RGD. The degree of substitution of the backbone with RGD, and the size of the poly(aspartic acid) polymer produced by the aminolysis, is controlled by adjusting the pH of the reaction mixture, the amount of RGD-linker present in the reaction mixture, the time of reaction, and the like.

The resulting RGD-poly(aspartic acid) polyvalent presenter is screened to determine the effect of the polyvalent presenter on fibrinogen binding to platelet membrane glycoprotein IIb/IIIa complex according to methods known in the art, e.g., platelet aggregation assays.

Example 3 – Polyvalent Polymeric Galactosides in Prevention of Adhesion of Ricins to Erythrocytes

Abbreviations.

PBS – phosphate buffered saline; Et – ethyl; MeOH – methanol; h – hour; i-Pr – isopropyl; pBMA – poly(butadiene-co-maleic acid); RCA – Risinus Communis Agglutinin; BHA – Bromelain-cleaved Hemagglutinin; LDH – lactate dehydrogenase.

General Procedure.

All common chemicals were used as received from suppliers without further purification, unless otherwise noted. Unless otherwise specified, all chemicals were purchased from Aldrich Chemical Co., St. Louis, Missouri. Red blood cells (RBCs or erythrocytes) from 2-week-old chicks were purchased from Spafas Inc. The erythrocytes, which were provided as a suspension (~5% v/v) in a storage buffer, were washed 4 times with phosphate buffered saline (PBS; 137 mM NaCl, 2.68 mM KCl, 7.75 mM Na_2HPO_4, 1.47 mM KH_2PO_4), pH 7.2 and then re-suspended in PBS (~0.5% v/v). Ricins (RCA_{120}, RCA_{60}) including fluorescein isothiocyanate (FITC)-labeled ricins (FITC-labeled RCA_{120}, FITC-labeled RCA_{60}) were purchased from Sigma Co.

Synthesis of Gal-β_O-L_1NH_2 and Gal-α_C-L_2NH_2 (Scheme 1):

Gal-β_O-L_1NH_2: To a solution of methylene chloride (180 mL) containing β-D-galactose pentaacetate (7.8 g, 19.98 mmol) and allyl alcohol (5.6 ml, 58.82 mmol) cooled in ice bath BF_3•Et_2O (4.0 mL, 32.52 mmol) was added dropwise. After stirring (4 h, 0 °C; then 30 h, about 20 °C), the mixture was poured into cold saturated $NaHCO_3$ (200 mL) in a separatory funnel. After shaking, the organic layer was separated out, and washed again with cold saturated $NaHCO_3$ (200 mL). After drying over $MgSO_4$, the methylene chloride solution was evaporated *in vacuo* to yield a pale yellow oil (R_f = 0.57 in 5% MeOH/CH_2Cl_2). The crude products were dissolved in methanol (100 mL), followed by addition of LiOH (2.88 g, 120 mmol) in water (50 mL). After stirring (12 h, about 20 °C), the reaction mixture was neutralized by adding 6.0 M HCl (about 20 mL). The aqueous mixture was concentrated *in vacuo* to afford a thick oily residue, which was purified with flash column chromatography (silica gel; 5% to 40% MeOH/CH_2Cl_2). The product, Gal-β_O-$CH_2CH{=}CH_2$, was obtained in 91% (4.0 g) yield over two steps as a pale yellow

oil (Rf= 0.80 in 30% MeOH/CH_2Cl_2). ^{1}H-NMR (300.1 MHz, CD_3OD): δ (ppm) 6.00-5.89 (m, 1H), 5.36-5.29 (dd, J = 17.2, 3.2, 1H), 5.18-5.12 (dd, J = 12.1, 3.2 Hz, 1H), 4.40-4.34 (dd, J = 13.0, 5.2 Hz, 1H), 4.27-4.25 (d, J = 7.3 Hz, 1H; H_{1ax}), 4.17-4.10 (dd, J = 13.0, 6.1 Hz, 1H), 3.90-3.83 (m, 1H), 3.80-3.68 (m, 3H), 3.56-3.50 (m, 2H); FAB-MS (glycerol): *m/z* 221 $[M+H]^+$; HRMS: calcd for $C_9H_{17}O_6$ 221.1024, found 221.1025.

A solution of Gal-β_O-CH_2CH=CH_2 (4.0 g, 18.17 mmol), $HSCH_2CH_2NH_2$•HCl (6.19 g, 54.5 mmol), and 4,4'-azobis(4-cyanovaleric acid) (0.4 g, 1.43 mmol) in water (50 mL)-methanol (5 mL) was degassed for 10 min *in vacuo* prior to being saturated with N_2 (by bubbling N_2 gas through the solution for 30 min). A reaction flask containing the mixture was placed in a photochemical reactor (Rayonet®), and was irradiated at 254 nm for 10 h. The irradiated mixture was neutralized by adding 2.0 M NaOH (28 mL), and immediately evaporated to remove volatiles. The evaporation yielded a pale yellow oil, which was purified with flash silica gel chromatography (10% MeOH/CH_2Cl_2 to 5% *i*-$PrNH_2$/40% MeOH/CH_2Cl_2). The adduct (Gal-β_O-L_1NH_2) was obtained as an oil (Rf= 0.33 in 5% *i*-$PrNH_2$/30% MeOH/CH_2Cl_2). ^{1}H-NMR (250.1 MHz, CD_3OD): δ (ppm) 4.22-4.19 (d, J = 7.4 Hz, 1H; H_{1ax}), 4.0-3.95 (ddd, J = 9.9, 6.1, 6.1 Hz, 1H), 3.87-3.81 (m, 1H), 3.78-3.63 (m, 4H), 3.53-3.43 (m, 2H), 2.81-2.76 (t, J = 6.5 Hz, 1H), 2.67-2.59 (q, J = 7.1 Hz, 4H), 1.92-1.82 (quin, J = 6.6 Hz, 2H); ^{13}C-NMR (100.6 MHz, CD_3OD): ? (ppm) 76.6, 75.8, 72.5, 70.3, 69.2, 62.5, 41.6, 35.6, 31.2, 29.0; FAB-MS (glycerol): *m/z* 298 $[M+H]^+$; HRMS: calcd for $C_{11}H_{24}NO_6S$ 298.1323, found 298.1324.

Gal-α_C-L_2NH_2: Gal-α_C-CH_2CH=CH_2 was prepared by α-*C*-allylation of β-D-galactose pentaacetate (Giannis, A.; Sandhoff, K. *Tetrahedron Lett.* **1985**, *26*, 1479-1482), and subsequent hydrolysis of the acetates. ^{1}H-NMR (400.0 MHz, CD_3OD): δ (ppm) 5.91-5.83 (m, 1H), 5.13-4.99 (m, 2H), 4.00-3.96 (m, 2H), 3.95-3.88 (m, 1H), 3.76-3.67 (m, 4H), 2.49-2.34 (m, 2H); ^{13}C-NMR (100.6 MHz, CD_3OD): δ (ppm) 136.7, 116.9, 75.7, 74.0, 71.9, 70.1, 70.0, 62.0, 31.0; FAB-MS

(glycerol): *m/z* 227 $[M+Na]^+$. Gal-α_C-L_2NH_2 was prepared from Gal-α_C-$CH_2CH=CH_2$ as described above. ^{1}H-NMR (400.0 MHz, CD_3OD): δ (ppm) 3.95-3.63 (m, 7H), 3.12 (t, J = 6.9, 2H), 2.81 (t, J = 3.4 Hz, 2H), 2.66-2.59 (m, 2H), 1.81-1.62 (m, 4H); ^{13}C-NMR (100.6 MHz, CD_3OD): δ (ppm) 75.7, 74.0, 71.9, 70.4, 70.2, 62.5, 39.9, 32.1, 26.8, 24.8, 20.9; FAB-MS (glycerol): *m/z* 282 $[M+H]^+$; HRMS: calcd for $C_{11}H_{24}NO_5S$ 282.1375, found 282.1374.

Synthesis of pAA(Gal-β), pBMA(Gal-β), and pBMA(Gal-α) (Figure 5): pAA(Gal-β): the method described below for synthesis of pAA(Gal-β; 0.4) is a protocol for general synthesis of pAA(Gal). To a solution of *N,N*-dimethylformamide (DMF, 8 mL) containing poly(*N*-acryloyloxysuccinimide) or pNAS (500 mg, equivalent to 3 mmol of NAS) (Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 4179-4190) was added Gal-β_O-L_1NH_2 (356 mg, 1.2 mmol) dissolved in DMF (2 mL), followed by addition of *i*-Pr_2NEt (0.2 mL, 1.2 mmol). After stirring (2 d, about 20 °C), the mixture was basified by adding 1.0 M NaOH (3 mL), followed by stirring for additional 2 h at about 20 °C. At the conclusion of the reaction, the mixture was transferred into a dialysis bag (MW cutoff about 12-14 kDa; Spectrum® from Sprcturm Medical Industries, Inc.), and was dialyzed at about 20 °C over 3 d: H_2O (2 x 4L), 0.05 M NaOH (4L), 0.5 M NH_4Cl (4L), and H_2O (2 x 4L). The content of the bag was lyophilized to afford pAA(Gal-β; 0.4) which was obtained as a fluffy white solid (499 mg). ^{1}H-NMR (500.1 MHz, D_2O): δ (ppm) 4.3 (d, J = 7.5 Hz; H_{1ax}), 3.89 (br s), 3.85 (s), 3.8-3.5 (m), 3.4 (br m), 3.3 (br s), 2.6 (br s), 2.2-1.9 (br d), 1.7-1.3 (br m); % S: calcd for pAA(Gal-β; 0.4) 6.97, found 6.71. Other pAA(Gal-?)s were prepared following the same procedures. % S: calcd for pAA(Gal-β; 0.2) 5.00, found 5.12; calcd for pAA(Gal-β; 0.6) 8.02, found 8.06; calcd for pAA(Gal-β; 0.8) 8.67, found 8.58; calcd for pAA(Gal-β; 1.0) 9.11, found 9.05.

Synthesis of pBMA(Gal-β) and pBMA(Gal-α):

These polymers were synthesized following the above protocol with a slight difference, in which poly(butadiene-*co*-maleic anhydride) or pBMAn was used instead of pNAS as a precursor polymer. An aliquot of pBMAn, which was provided as a solution in acetone (Polysciences, Inc.), was dried *in vacuo* and redissolved in DMF

before use. pBMA(Gal-β; 0.09): ^{1}H-NMR (500.1 MHz, D_2O): δ (ppm) 5.6 (br s), 5.4 (br s), 3.9-3.5 (br m), 3.4-3.3 (br m), 3.0-2.9 (br m), 2.7-2.5 (br m), 2.4-2.1 (br s), 1.8 (br s), 3.9-3.5 (br m). pBMA(Gal-α; 0.09): ^{1}H-NMR (500.1 MHz, D_2O): δ (ppm) 5.6 (br s), 5.5-5.3 (br d), 4.3-3.7 (br m), 3.6 (br m), 3.3 (br s), 2.9 (br s), 2.8-2.4 (br s), 2.4-2.0 (br s), 1.8-1.4 (br m), 1.0 (br s). % S: calcd for pAA(Gal-α; 0.05) 1.62, found 1.55; calcd for pAA(Gal-α; 0.09) 2.62, found 2.69; calcd for pAA(Gal-α; 0.17) 4.11, found 4.12; calcd for pAA(Gal-α; 0.22) 4.81, found 4.85.

Ricin-mediated Agglutination of Chick RBCs, and Its Prevention by pAA(Gal): (i) Adhesion of ricin to chick RBCs: A suspension of RBCs (0.5% v/v; 0.4 mL) in PBS, pH 7.2 was mixed well with PBS solution of fluorescent ricin (0.4 mL of FITC-labeled RCA_{120} (40 nM) or FITC-labeled RCA_{60} (1.4 μM)) contained in an 1-mL Eppendorf vial. After incubation for 2 h at 4 °C, the mixture was centrifuged for 2 min at 2000 rpm. After discarding a supernatant solution, red pellets were washed with 1.0 mL of PBS, and resuspended gently in PBS (0.2 mL). Optical images of absorption and fluorescence of RCA-adsorbed RBCs were obtained by taking an aliquot of suspended pellets on a glass slide, and examining the sample with optical and fluorescence microscopy (Leica DMRX).

(ii) Protection of RBCs by pAA(Gal-β) from the cellular adsorption of ricin: A solution of FITC-labeled RCA_{120} (80 nM; 0.2 mL) in PBS was mixed with a PBS solution (0.2 mL) of pAA(Gal-β; 0.4) (90 ?g mL^{-1} or [Gal] equal to about 200 μM) in an Eppendorf vial. After incubation (30 min, 4 °C), the ricin-polymer mixture was added to a suspension of RBCs (0.5% v/v; 0.4 mL) in PBS, followed by gentle agitation and incubation for 2 h at 4 °C. The incubated mixture was centrifuged for 2 min at 2000 rpm. Red pellets, obtained after removing the supernatant, were washed with 1.0 mL of PBS, and resuspended in 0.2 mL of PBS before being examined with an optical microscope.

Hemagglutination (ricin-induced) Inhibition (HAI) Assay:

The titer of the prepared PBS solution of ricins (RCA_{120} = 16 nM; RCA_{60} = 1.9 μM) was determined by 2-fold serial dilution of 50 μL of the ricin solution (mg mL^{-1}) through 12 wells (8 x 12-well microtiter plate with conically-shaped bottoms; ICN

Flow). Another 50 μL PBS was added to each well, followed by addition of a suspension of chicken erythrocytes in PBS (100 μL). The solution was mixed and incubated at about 20 °C for 1 h. The end point of hemagglutination (HA) is defined as the last well in which a sufficient amount of ricin remains to agglutinate the erythrocytes. A stock PBS solution (50 μL) of polymeric galactoside (1-2 mg mL^{-1}; [Gal] about 2-6 mM) or monovalent galactoside (5 mM) was 2-fold serially diluted through 12 microtiter wells containing 50 μL of PBS. After serial dilutions of solutions of polymeric or monomeric galactoside, each well (50 μL) was mixed with 50 μL of RCA_{120} (16 nM) or RCA_{60} (1.9 μM). After 30 min of incubation at about 20°C, 100 μL of a suspension of chicken erythrocytes (0.5% v/v) was added to each well followed by gentle agitation and incubation (1 h, about 20 °C). The end point of HAI is the last well in which an agglutinated pellet is observed. This end point (K_i^{HAI}) is defined as the lowest concentration of galactoside in solution that inhibited the ricin-induced agglutination of erythrocytes. The values of K_i^{HAI} were calculated on the basis of at least 5 independent trials.

Results and Discussion

Synthesis of Galactoside-Presenting Polymers:

Two derivatives of D-galactosides (Gal-β_O-L_1NH_2, Gal-α_C-L_2NH_2) were synthesized as monomeric precursors to polymeric polyvalent D-galactosides: Gal-β_O-L_1NH_2 contains a β-*O*-linkage between the galactoside (Gal) group and the amine-terminated linker (Scheme 1); Gal-α_C-L_2NH_2 contains an α-*C*-glycoside. As a C_1-epimeric analog of β-*O*-galactosides, the Gal-α_C- L_2NH_2 was chosen because it could be prepared easily in high stereoselectivity and large scale. The *C*-glycosidic linkage of Gal-α_C- L_2NH_2 provides the additional advantage of resistance to chemical and enzymatic hydrolysis. Both epimers of D-galactoside were used to compare their binding affinities to ricins. Two types of polymers—poly(acrylic acid) (pAA; M_W = ~ 140 kDa, M_W/M_n = 1.91) (Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 4179-4190) and poly(butadiene-*co*-maleic acid) (pBMA; M_W = 10-15 kDa)—were used as polymeric scaffolds to present multiple copies of monovalent

galactosides as amide side chains. These polymers were referred to as pAA(Gal-β), pAA(Gal-α), pBMA(Gal-β), and pBMA(Gal-α). The pAA-based polymers were chosen with the expectation that they would be relatively flexible, at least at high ionic strength; the pBMA-derived polymers were expected to be less flexible. Synthesis of pAA(Gal-β) was achieved using methods described previously by allowing poly(*N*-acryloyloxysuccinimide) (pNAS) (Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 4179-4190) to react with Gal-βO- L_1NH_2 in DMF (about 20 °C, 2 d), and quenching with excess 1.0 M NaOH. By allowing pNAS to react with χ equivalents of Gal-βO-L_1NH_2 per equivalent of active ester groups on the polymer (χ = 0.2, 0.4, 0.6, 0.8, and 1.0), various pAA's (Gal-β; 0.2 to 1.0) were prepared. The parameter χ is also equivalent to the mole fraction of Gal in the polymers, and is defined as the number of side chains containing Gal divided by the total number of side chains (Scheme 1). The same strategy was applied to poly(butadiene-*co*-maleic anhydride) (pBMAn) generated pBMA(Gal-β; 0.05 to 0.22) and pBMA(Gal-α; 0.05 to 0.22). All the polymers were purified by dialysis (MW cutoff ~ 3.5 kDa), and characterized by means of ^{1}H-NMR spectroscopy and combustion analysis (sulfur). The yields of amide-forming reactions were ≥95% and ≥65% for pNAS and pBMAn, respectively, on the basis of the combustion analysis of the polymers.

Inhibition of Adhesion of Ricins to Erythrocytes by Galactoside-Presenting Polymers: Red blood cells (RBCs) from 2-week-old chick were used as a model system of mammalian cells. Erythrocytes lack a nucleus, and do not synthesize proteins. Nevertheless, they provide a good model of the cells targeted by ricin, and provide a system with which to study the adhesion of ricin to the cell surface: the surface of erythrocytes presents a variety of β-galactoside-containing glycoconjugates (about 2-3 x 10^6 Gal residues per human RBC) (Sandvig, K.; Olsnes, S.; Pihl, A. *J. Biol. Chem.* **1976**, *251*, 3977-3984).

Representative absorption and fluorescence images were obtained and demonstrated that ricins attached to chick RBCs and caused their aggregation and lysis. Labeling the ricins with fluorescent isothiocyanate (FITC) established that aggregation and lysis were due to the action of the ricins. It was also demonstrated that a polyvalent galactoside, pAA(Gal-β; 0.4) prevented these effects.

Using chick erythrocytes (0.5% by volume) as a suspension in phosphate buffered saline

(PBS) solution, pH 7.2 and ricins (RCA_{120} ~ 16 nM; RCA_{60} ~ 1.9 μM), the activities of polymeric galactosides in inhibiting ricin-mediated agglutination were assayed. Table 10 summarizes the hemagglutination inhibition (HAI) activity, K_i^{HAI} (defined as the lowest concentration of an inhibitor required to prevent hemagglutination) of purified polymers with various mole fractions of carbohydrate-containing side chains ($\chi^{carbohydrate}$). [a] The value of K_i^{HAI} refers to the lowest concentration of carbohydrate-containing side chains of the polymer that could inhibit ricin-induced agglutination of chick erythrocytes. Each value represents an average of five independent measurements; the experimental uncertainty in each value is approximately ±50%. [b] No inhibition was observed at the indicated concentrations. [c] This value represents the concentration of carboxylic acid side chains of the polymer in solution that could inhibit ricin-induced agglutination of chick erythrocytes.

Table 10. Inhibition of ricin-induced agglutination of chick erythrocytes by polyvalent polymeric galactosides

Inhibitor	K_I^{HAI} (μM)[a]		Inhibitor	K_I^{HAI} (μM)[a]	
	RCA_{120}	RCA_{60}		RCA_{120}	RCA_{60}
Gal-β-Ome	200	42	pBMA(Gal-β; 0)	>30000[b,c]	≥30000[b,c]
Gal-α-Ome	400	50	pBMA(Gal-β; 0.05)	2.0	1.0
Gal-β$_O$-L_1NH_2	37	16	pBMA(Gal-β; 0.09)	14	12
Gal-α$_C$-L_2NH_2	250	39	pBMA(Gal-β; 0.17)	5.0	>80
GlcNAc-β$_O$-L_1NH_2	>9000[b]	>9000[b]	pBMA(Gal-β; 0.22)	0.73	≥94
			pBMA(Gal-α; 0.05)	>160	≥160
PAA(Gal-β; 0)	>35000[b,c]	>35000[b,c]	pBMA(Gal-α; 0.09)	280	33
PAA(Gal-β; 0.2)	0.16	8.1	pBMA(Gal-α; 0.17)	61	17
PAA(Gal-β; 0.4)	0.14	17	pBMA(Gal-α; 0.22)	12	5.0
PAA(Gal-β; 0.6)	0.18	20	pAA(GlcNAc-β; 0.2)	>290[b]	>290[b]
PAA(Gal-β; 0.8)	0.27	42	pAA(NeuAc-α; 0.2)	>170[b]	>170[b]
PAA(Gal-β; 1.0)	0.56	180	pBMA(NeuAc-α; 0.2)	>200[b]	>200[b]

Monovalent Gal-βO-L_1NH_2 gave values of K_i^{HAI}, 5- and 3-fold lower than those of Gal-β-OMe, against RCA_{120} and RCA_{60}, respectively. Monovalent galactosides containing a β-*O*-anomeric configuration gave activities better than the corresponding β-galactosides, though the difference was not great. This observation implied that binding of Gal residues to ricin Gal-binding sites was not highly sensitive to the anomeric configuration (β ≥ α) or to the nature of atom (O ≥ C) attached to the anomeric carbon.

pAA(Gal-β; 0.2 to 1.0) showed HAI activity, K_i^{HAI}, against RCA_{120}-induced agglutination at submicromolar concentrations of Gal moieties in solution. The same polymers had HAI activities against RCA_{60} that were about 50- to about 300-fold lower than those against RCA_{120}; the activities against RCA_{60} were, at best, 50 times better than those of monomeric Gal derivatives. By contrast, pAA(Gal-β; 0.4) had an inhibitory activity (against RCA_{120}) that was about 1500 times higher than that of monovalent Gal-β-OMe, and about 270 times higher than that of Gal-βO-L_1NH_2. pBMA(Gal-β; 0.05 to 0.22) showed HAI activities at (sub)micromolar concentrations against RCA_{120} and RCA_{60}; the activities against RCA_{120} were better than those against RCA_{60}. pBMA(Gal-α; 0.05 to 0.22) had, however, HAI activities against RCA_{60} approximately 4 times better than those against RCA_{120}. Of the polyvalent galactosides built on pBMA, pBMA(Gal-β; 0.22) and pBMA(Gal-β; 0.05) were the most active inhibitors against RCA_{120} and RCA_{60}, respectively. pBMA(Gal)s are relatively small polymers with molecular weight about 10-15 kDa, and have a polymer backbone (pBMA) that is biocompatible (Conroy, C. W.; Wynns, G. C.; Maren, T. H. *Bioorg. Chem.* **1996**, *24*, 262-272).

Other polymers were tested presenting multiple copies of non-galactoside carbohydrates (pAA(NeuAc-α; 0.2) (Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 4179-4190), pBMA(NeuAc-α; 0.2 to 1.0), pAA(GlcNAc-β; 0.2 to 1.0)) as controls: none of them inhibited the agglutination of erythrocytes by ricin at concentrations comparable to those in Table 10 (K_i^{HAI} > 300 μM). Thus, the conclusion was reached that ricin-induced

agglutination of erythrocytes was inhibited selectively by polymers presenting galactosides. Both α and β anomers seemed to be approximately equal in effectiveness. These results suggested that the inhibition by polymeric galactosides was primarily due to the specific binding of galactoside ligands to the Gal receptor sites of ricins. We believe that the high activity of polymeric polyvalent galactosides relative to monovalent galactosides is partly due to multivalent (entropically-enhanced) binding of galactoside ligands to multiple receptor sites.

Figure 6 summarizes the HAI activity of the polymers against ricins. Figure 6a (with RCA_{120}) shows that values of K_i^{HAI} of polymeric galactosides are a non-linear function of the mole fraction of Gal (χ^{Gal}) of the polymers. The relationship between activity and χ^{Gal} of the polymers in inhibiting hemagglutination by RCA_{60} seem to be related closely to that describing inhibition by RCA_{120}. The K_i^{HAI} - χ^{Gal} relationship of the polymers depended also on the type of polymer backbone: a smooth curve with large, flexible pAA(Gal-β); a sharp, partly quasi-linear relationship with small, extended pBMA(Gal). pAA(Gal-β; 0.01) (degree of polymerization = DP ~ 2000), presenting a low density of Gal side chains (about 20 Gals per polymer chain), still showed an activity (K_i^{HAI} ~ 2.0 μM), that was greater than that of monovalent Gal-β-L_1NH_2 by a factor of 19. We ascribe the increased activity of pAA(Gal-β; 0.01) relative to monomeric Gal-β-L_1NH_2 to the flexibility of its polymer backbone, and thus to its ability to adjust to the distances between galactoside ligands; this flexibility facilitates multivalent binding to the receptor sites of ricin.

Under the conditions we used in assaying the Gal-presenting polymers for their ability to inhibit agglutination, the titer of RCA_{120}—a minimal concentration that could agglutinate RBCs (200 μL of 0.25% by volume, suspended in PBS solution)—was 4 nM, and that of RCA_{60} was 480 nM. This difference indicated that RCA_{60} (one B-chain; about 3 Gal receptor sites) was more weakly agglutinating than is RCA_{120} (two B-chains; about 6 Gal receptor sites). Monovalent galactosides were more effective against RCA_{60} than against RCA_{120} in preventing the ricin-induced agglutination of erythrocytes. Since the inhibition of agglutination reflects the

competitive binding of monovalent galactosides to the Gal receptor sites of ricin, this difference in activities suggested at least two hypotheses regarding adhesion of ricins to cells. First, the difference in the observed HAI activities of monomeric galactosides against RCA_{60} and against RCA_{120} might be due to differences in the intrinsic affinities of monomeric galactosides for Gal receptor sites on RCA_{60} and RCA_{120}. Binding studies of several monomeric derivatives of galactosides with the two ricins using equilibrium dialysis and fluorescence techniques, however, showed that the two ricins had similar affinities for the monovalent galactosides (within a factor less of than 10) (Baenziger, J. U.; Fiete, D. *J. Biol. Chem.* **1979**, *254*, 9795-9799: Houston, L. L.; Dooley, T. P. *J. Biol. Chem.* **1982**, *257*, 4147-4151; Rivera-Sagredo, A.; Solis, D.; Diaz-Mauriño, T.; Jimenez-Barbero, J.; Martin-Lomas, M. *Eur. J. Biochem.* **1991**, *197*, 217-228). Second, the low values of K_i^{HAI} of monovalent galactosides against RCA_{60} relative to RCA_{120} may be related to the number (n) of blocked Gal receptor sites of ricins required for inhibition of agglutination relative to the total number of Gal receptor sites of ricins: n/3 (RCA_{60}); n/6 (RCA_{120}). That is, blocking of the same number of Gal sites on RCA_{60} and RCA_{120} results in a larger fraction of Gal sites on RCA_{60} being blocked, and is more effective in abolishing the agglutination ability of RCA_{60} than of RCA_{120}.

Polymeric galactosides gave values of K_i^{HAI} that were lower for RCA_{120} than for RCA_{60}. The relative enhancement of the activities of galactoside moieties on polyvalent presentation (that is, the value of K_i^{HAI} for the polymer relative to that of the monomer) was higher for RCA_{120} than for RCA_{60}, as well. These results suggest that polyvalent ligands are more effective as inhibitors against a target with a higher valency (RCA_{120}) than against one with a lower valency (RCA_{60}).

With RCA_{60}, some of the polymers (pAA(Gal-β; 0.6 to 1.0), pBMA(Gal-β; 0.17, 0.22), pBMA(Gal- α; 0.05)) were less effective in blocking ricin-mediated agglutination (on a per Gal basis) than were the corresponding monovalent galactosides. These results support the conclusion that, for some polymeric polyvalent inhibitors, there may not be significant entropic enhancement of binding to

the small number of receptor sites presented by BHA (3 receptor sites), LDH (4 receptor sites) and RCA_{60} (about 3 receptor sites) and that ligands presented on polymer backbone may have lower affinities for a receptor than do these ligands without the encumbering polymer (possibly due to undefined interactions between the polymer backbone and the protein).

The fact that many of the polymers were more effective in blocking ricin-mediated agglutination (on a per Gal basis) than were the corresponding monovalent galactosides suggests that factors other than the fractional occupancy of receptor sites determine K_i^{HAI}. Since each polymer presents multiple Gal moieties, the binding of polymer to ricin may, of course, be relatively tight, even if individual Gal groups bind relatively weakly. The experiments presented here do not directly measure the occupancy of the Gal receptor sites on ricin, and the inhibition of hemagglutination may therefore be due either to entropically-enhanced binding of these Gal-binding receptor sites, or to other, non-receptor directed effects such as steric inhibition.

Certain naturally occurring, structurally complex glycoproteins and oligosaccharides bind tightly to B-chains of ricins. Glycopeptides derived enzymatically from fetuins (a class of glycoproteins presenting a variety of carbohydrate clusters) are one example: these compounds have dissociation constants to ricins (and to B-chains) of $K_d \sim 10$ to 0.1 μM (Baenziger, J. U.; Fiete, D. *J. Biol. Chem.* **1979**, *254*, 9795-9799). These glycopeptides are multi-branched oligosaccharides and oligopeptides that present multiple copies of galactosides and *N*-acetylgalactosamine moieties. The galactoside-containing glycoconjugates are, thus, structurally multivalent in terms of the number and intramolecular distribution of galactosides per molecule, and we believe that these structural features may account for the high binding affinities of these glycopeptides to ricins relative to monomeric galactosides. Our galactoside-presenting polymers, pAA(Gal) and pBMA(Gal), are synthetically well-characterized, polyvalent species with the hemagglutination inhibition activities, $K_i^{HAI} \sim$ up to 0.1 μM. These polymers can be synthesized conveniently, and especially those presenting *C*-galactosides are relatively stable chemically and enzymatically, compared to the fetuin-derived, enzyme-sensitive

glycopeptides.

In summary, the above results demonstrate that synthetic polymers presenting multiple copies of a simple derivative of β-D-galactose as side chains effectively inhibited adhesion of ricins to chicken erythrocytes as examined with optical/fluorescence microscopy, and as measured quantitatively by the hemagglutination inhibition (HAI) assay. Upon conversion of monovalent galactosides—which are themselves weak inhibitors of ricin-cell adhesion—to polymeric polyvalent galactosides, the HAI activities of the polymer-bound galactoside residues increased by factors of about 10^2 to 10^3 for RCA_{120}, but only by 50 for RCA_{60}, calculated on the basis of the total concentration of these sugar units in the assay solution. The activities of the polymeric galactosides were affected by a number of factors such as the mole fraction of Gal-containing side chains of the polymer, the C-1 epimeric configurations ($\beta \geq \alpha$) of galactosides, and the polymer backbone.

Example 4 – Polyvalent Polymeric N-acetylglucosamine Induction of Acrosomal Exocytosis in Mouse Sperm

Abbreviations: Gal-transferase, β-1,4-GalactosylTransferase; ZP, zona pellucida; GlcNAc, *N*-acetylglucosamine; Gal, galactose; pA, poly(acrylamide); pAA, poly(acrylic acid); pAA(GlcNAc-β), poly(acrylic acid) presenting multiple copies of β-*O*-linked GlcNAc as amide side chains; pAA(Gal-β), poly(acrylic acid) presenting multiple copies of β-*O*-linked Gal as amide side chains.

Materials and Methods

Polymeric polyvalent *N*-acetylglucosamine (GlcNAc) and galactoside (Gal): Poly(acrylic acid) presenting multiple copies of GlcNAc or Gal as amide side chains—pAA(GlcNAc; χ) and pAA(Gal; χ): χ = 0.2, 0.4, 0.6, 0.8, 1.0—were prepared according to procedures similar to those described above in Example 3 for the preparation of the polymeric polyvalent compounds. Figure 7 depicts the structure of polyvalent polymeric *N*-acetylglucosamine (GlcNAc) and galactoside (Gal). The pAA(GlcNAc) and pAA(Gal) refer

to poly(acrylic acid) presenting multiple copies of GlcNAc-β-L_1-NH_2 and Gal-β-L_1-NH_2, respectively, as amide side chains.

Procedures for acrosome reactions of mouse sperm:

Mouse sperm were capacitated for 30 min at 35°C, and incubated at 37°C in the presence or absence of polymers. Each experiment had the following controls: 1) phosphate buffer used only to assess the degree of "spontaneous" acrosome reactions (usually less than 5% during the assay and was subtracted from all other determinations); 2) polymer backbone—poly(acrylyl amide), or pA — used at the same mass as that used to generate the desired molarity of GlcNAc in pAA(GlcNAc); 3) zona pellucida glycoproteins (ZP), the "native" inducer; and 4) calcium ionophore A23187 used to drive the reaction mixture at time zero, 15 min, 30 min, and sometimes 60 min. The values (%) of acrosome-reacted sperm by ZP and the ionophore were 27.1 (±0.10) and 53.7, respectively. Poly(acrylamide), or pA was tested as a negative control: the values of the x-axis indicate the concentrations of carboxamide groups of pA. The sperm sample was dried onto slides, stained for the intact acrosome, and the number of stained vs. unstained cells were determined in 200 sperm for each sample.

Results and Discussion

Induction of acrosomal exocytosis by multivalent GlcNAc:

Figure 8 summarizes the activities (acrosome-reacted sperm, %) of a monovalent GlcNAc and a polyvalent GlcNAc in induction of acrosome reaction. The pAA(GlcNAc; 0.2) induced significant amounts (9-17%) of acrosome reaction at concentrations of 100 μM ([GlcNAc]), while monovalent GlcNAc or polymer backbone (pA) did not induce meaningful amounts of acrosome reaction (<5%) under the identical experimental conditions. This example illustrates that the acrosome reaction of sperm appears to require multivalent binding (spatial aggregation on the surface) of Gal-transferase to GlcNAc residues of egg coat, and can be induced by a synthetic polymer presenting multiple copies of a simple *O*-linked derivative of GlcNAc.

Figure 9a summarizes the activities (acrosome-reacted sperm, %) of a series of pAA(GlcNAc; χ) (χ = 0.2, 0.3, 0.6, and 1.0) and pAA(Gal; χ)(χ = 0.3, 0.6, and 1.0) in induction of acrosome reaction of mouse sperm. Polymers of pAA(GlcNAc) showed weak to moderate activities (4-10%) at 0.1-100 μM ([GlcNAc]). Above 100 μM, they showed

increased activities (17-27%). Additionally, the results indicate that the activities (acrosome-reacted sperm, %) of pAA(GlcNAc) increased sharply at around 100 μM of GlcNAc. Figure 9a also shows the activities of poly(acrylic acid) presenting O-linked galactoside, pAA(Gal). The low (no) activities were probably due to low (no)-affinity binding of Gal residues of pAA(Gal) to Gal-transferase.

Figure 9b is a plot of the activities (acrosome-reacted sperm, %) of the pAA(GlcNAc; χ)(χ = 0.2, 0.3, 0.6, and 1.0) at 100 μM ([GlcNAc]) versus mole fraction (χ) of GlcNAc of pAA(GlcNAc). The plot is derived from Figure 9a and 14% for pAA(NeuAc; 1.0) is an estimated value. The plot utilizes the concentration of 100 μM ([GlcNAc]), because it is a threshold concentration leading to large increases in the activities of pAA(GlcNAc). The correlation between the activities and χ is non-linear; (a) there is a sharp increase in the activities between χ = 0 and χ = 0.2; (b) after reaching at a maximum (23%) activity, the activities decrease gradually.

Figure 10 summarizes the activities of inhibition of sperm-egg binding by polyvalent polymeric GlcNAc. Three polymers—pAA(GlcNAc; χ = 0.3, 0.6, and 1.0)—, were tested at 100 μM ([GlcNAc]) for *in vitro* activity in inhibition of sperm-egg binding and reduced the number of sperm (bound per egg) from 26 (control, and pA) to ~10, 5, and 5. The results suggest that the polymeric polyvalent GlcNAc induced acrosome reaction in mouse sperm and inhibit sperm-egg binding. The polymer backbone, pA, was used as a control at 100 μM ([-$CONH_2$]).

Conclusions

(1) pAA(GlcNAc) induced acrosomal exocytosis, while monovalent GlcNAc did not; polyvalency is involved in the acrosome process.

(2) pAA(Gal) that present non-specific ligand to Gal-transferase was not effective in inducing acrosomal exocytosis.

(3) There was a non-linear relationship between the activities (acrosome-reacted sperm, %) and mole fraction (χ) of pAA(GlcNAc) at certain concentration of GlcNAc.

(4) pAA(GlcNAc) inhibited sperm-egg binding; the mechanism may involve the acrosome reaction of sperm by the polyvalent GlcNAc.

Example 5: Generation of pMVMA(NeuAc) using quasi-solid phase reaction (*see*, Figure 11a)

Solutions of co-polymers of poly(methyl vinyl ether-*co*-maleic acid)(NeuAc-L_1), or pMVMA(NeuAc-L_1) were prepared by reacting of RNH_2 ($NeuAc\text{-}L_1NH_2$) with poly(methyl vinyl ether-*co*-maleic anhydride), or pMVMAn using different molar equivalent (mol equiv) of RNH_2 to anhydride groups of pMVMAn (mol equiv = {number of moles of RNH_2}/{number of moles of anhydride groups of pMVMAn}) and using aqueous solutions of amines adjusted to pH 12. Co-polymeric pMVMA(NeuAc) for which mol equiv is >0 was generated in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 3 mg of pMVMAn (M_n = 67000, 80000, 311000, 485000, or 1130000 $gmol^{-1}$) into a well; (ii) soaking the powder with a variable amount (10-38 mL) of 0.1 M of RNH_2 ($NeuAc\text{-}L_1NH_2$) in PBS buffer, pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each solution of co-polymers (pH ~ 3) generated in a well was neutralized to pH ~ 7 by adding 30 mL of 1.0 M NaOH and adjusted to 100 or 200 mL (total volume) with PBS, pH 7.2, before the inhibition assay of influenza virus-induced agglutination of chicken red blood cells.

Example 6: Generation of pMVMA(NeuAc;R) using quasi-solid phase reaction (*see*, Figure 11b)

The protocol used in Example 5 was extended to the preparation of ter-polymers pMVMA(NeuAc-L_1; R). A three-component mixture pMVMAn, $NeuAc\text{-}L_1NH_2$ and R_2NH_2(aliphatic amines, aromatic amines, amino acids, aminosugars, or peptides) was sonicated. Ter -polymeric pMVMA(NeuAc;R_2) for which mol equiv of $NeuAc\text{-}L_1NH_2$ and R_2NH_2 to anhydride group of pMVMAn are >0 was generated in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 3 mg of pMVMAn (M_n = 67000, 80000, 311000, 485000, or 1130000 $gmol^{-1}$) into a well; (ii) soaking the powder with 10 mL of 0.1 M $NeuAc\text{-}L_1NH_2$, and a variable amount (2-20 mL) of 0.1 M of RNH_2 (examples: naphthylalanine, phenylalanine, cyclohexylamine, phenylethylamine, 4-aminobenzoic acid, or mannosamine) in PBS buffer, pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each solution of ter-polymers (pH ~ 3) generated in a well was neutralized to pH ~ 7 by adding 30 mL of 1.0 M NaOH and adjusted

to 100 or 200 mL (total volume) with PBS, pH 7.2, before the inhibition assay of influenza virus-induced agglutination of chicken red blood cells.

Example 7: Generation of pAA(Gal) using quasi-solid phase reaction (*see*, Figure 11c)

Solutions of co-polymers of pAA(Gal) were prepared by reacting of RNH_2 (Gal-b-L_2NH_2; Gal-a-L_3NH_2) with poly(acrylic anhydride) (pAAn; M_n = 20700 $gmol^{-1}$, M_w = 39500 $gmol^{-1}$) using different molar equivalent (mol equiv) of RNH_2 to anhydride groups of pAAn (mol equiv = {number of moles of RNH_2}/{number of moles of anhydride groups of pAAn}) and using aqueous solutions of amines adjusted to pH 12. Co-polymeric pAA(Gal) for which mol equiv is >0 was generated in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 6 mg of pAAn into a well; (ii) soaking the powder with a variable amount (10-100 mL) of 0.1 M of RNH_2 (Gal-b-L_2NH_2, or Gal-a-L_3NH_2) in PBS buffer, pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each solution of co-polymers (pH ~ 3) generated in a well was neutralized to pH ~ 7 by adding 60 mL of 1.0 M NaOH and adjusted to 100 or 200 mL (total volume) with PBS, pH 7.2, before the inhibition assay of ricin-induced agglutination of chicken red blood cells.

Example 8: Generation of pBMA(Gal) using quasi-solid phase reaction (*see*, Figure 6d)

The protocol used in Example 7 was extended similarly to the preparation of co-polymers of poly(butadiene-*co*-maleic acid)(Gal), or pBMA(Gal). Solutions of co-polymers of pBMA(Gal) were prepared by reacting of RNH_2 (Gal-b-L_2NH_2; Gal-a-L_3NH_2) with poly(butadiene-*co*-maleic anhydride) (pBMAn; M_w = 10000-15000 $gmol^{-1}$) using different molar equivalent (mol equiv) of RNH_2 to anhydride groups of pBMAn (mol equiv = {number of moles of RNH_2}/{number of moles of anhydride groups of pBMAn}) and using aqueous solutions of amines adjusted to pH 12. Co-polymeric pBMA(Gal) for which mol equiv is >0 was generated in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 6 mg of pBMAn into a well; (ii) soaking the polymer with a variable amount (10-100 mL) of 0.1 M of RNH_2 (Gal-b-L_2NH_2, or Gal-a-L_3NH_2) in PBS buffer, pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each reaction mixture of co-polymers (pH ~ 3) generated in a well was neutralized to pH ~ 7 by adding 60 mL of 1.0 M NaOH and adjusted to 100 or 200 mL (total volume) with PBS, pH 7.2, before the inhibition assay of ricin-induced agglutination of chicken red blood cells.

Example 9: Generation of pAA(SLe^x) using quasi-solid phase reaction (*see*, Figure 12a)

The protocol used in Example 7 is extended similarly to the preparation of co-polymers of pAA(SLe^x). Solutions of co-polymers of pAA(SLe^x) are prepared by reacting RNH_2 (SLe^x-NH_2) with pAAn using different molar equivalent (mol equiv) of RNH_2 to anhydride groups of pAAn (mol equiv = {number of moles of RNH_2}/{number of moles of anhydride groups of pAAn}) and using aqueous solutions of amines adjusted to pH 12. Co-polymeric pAA(SLe^x) for which mol equiv is >0 is generated in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 6 mg of pAAn (M_n = 20700 $gmol^{-1}$, M_w = 39500 $gmol^{-1}$) into a well; (ii) soaking the polymer with a variable amount (10-100 mL) of 0.1 M of RNH_2 (SLe^x-NH_2) in PBS buffer, pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each reaction mixture of co-polymers (pH ~ 3) generated in a well is neutralized to pH ~ 7 by adding 60 mL of 1.0 M NaOH and adjusted to 100 or 200 mL (total volume) with PBS, pH 7.2, before the inhibition assay of adhesion of neutrophils to endothelial cells.

Example 10: Generation of pAA(Bacitracin;R) using quasi-solid phase reaction (*see*, Figure 12b)

The protocol used in Example 6 is extended to similarly to the preparation of ter-polymers pAA(Bacitracin;R). A three-component mixture including pAAn, Bacitracin and R_2NH_2(aliphatic amines, aromatic amines, amino acids, aminosugars, or peptides) is sonicated. Ter -polymeric pAA(Bacitracin;R) for which mol equiv of Bacitracin and RNH_2 to anhydride group of pAAn are >0 is generated in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 3 mg of pAAn (M_n = 20700 $gmol^{-1}$, M_w = 39500 $gmol^{-1}$) into a well; (ii) soaking the powder with 10 mL of 0.1 M Bacitracin, and a variable amount (2-20 mL) of 0.1 M of RNH_2 (examples: naphthylalanine, phenylalanine, cyclohexylamine, phenylethylamine, 4-aminobenzoic acid, or mannosamine) in PBS buffer, pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each solution of ter-polymers (pH ~ 3) generated in a well is neutralized to pH ~ 7 by adding 30 mL of 1.0 M NaOH and adjusted to 100 or 200 mL (total volume) with PBS, pH 7.2, before the inhibition assay of bacterial growth.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for preparing a polyvalent presenter for administration to a subject for treating a disease or condition, comprising:

(a) constructing and arranging a plurality of groups A on a framework forming a polyvalent presenter for treating a disease or condition, said construction and arrangement being made based on a therapeutic effect due to the blanketing of a collection of target binding sites B within a subject resulting from multivalent interaction of said groups A with a collection of target binding sites B, wherein said blanketing optionally results from the conformal interface interactions of a plurality of polyvalent presenters with collections of target binding sites B;

said method optionally further comprising one or more of the following steps:

(b) basing the construction and arrangement of the plurality of groups A on the framework on the ability of the polyvalent presenter to form a gel-like barrier surrounding the array of target binding sites B upon the conformal interface interaction of the polyvalent presenter with the collection of target binding sites B;

(c) basing the construction and arrangement of the plurality of groups A on the framework on the ability of the polyvalent presenter to interact with a known or predicted spatial arrangement of a collection of target binding sites B on an interface within the subject;

(d) selecting a type of group A which is a weak binder based on an analysis of the individual interaction between a single group A and a plurality of target binding sites B provided that a plurality of groups A meets a threshold collective binding upon the conformal interface interaction of the polyvalent presenter with a collection of target sites B;

(e) basing the construction and arrangement of the plurality of groups A on the framework on providing flexibility to the polyvalent presenter allowing it to conform to a collection of target binding sites B;

(f) selecting a type of framework for its ability to impart a desirable characteristic to the polyvalent presenter, wherein said desirable characteristic is optionally selected from the group consisting of flexibility and enhancement of the solubility of the polyvalent presenter.

2. The method of claim 1 wherein the plurality of Groups A are attached to a framework via a linker, said method optionally further comprising one or more of the following steps:

(a) selecting a type of linker based on its ability to impart flexibility to the polyvalent presenter;

(b) selecting a type of linker based on the ability of the linker to provide groups A to the collection of target binding sites B within a subject;

(c) selecting a length or type of linker based on a factor which affects the ability of the linker to provide groups A to the collection of target binding sites B within a subject, said factor optionally selected from the group consisting of the hydrophobicity, the hydrophilicity, and the diameter of the linker, wherein selection of said hydrophobicity or hydrophilicity of the linker is based on the hydrophobicity or hydrophilicity of an environment within the subject which provides access to a target binding site B and selection of said diameter of the linker is based on a known or predicted diameter of a channel within the subject which provides access to a target binding site B.

3. A method for treating a disease or condition, comprising:

administering to a subject a plurality of groups A, optionally in the form of a polyvalent presenter, such that the treatment of the disease or condition occurs by the conformal surface interaction of a polyvalent presenter with a collection of binding sites B within the subject, the conformal surface interaction resulting in the blanketing of a collection or array of target binding sites B within the subject.

4. Use of a plurality of groups A, optionally in the form of a polyvalent presenter, for the preparation of a medicament for the treatment of a disease or condition in a subject, such that the treatment of the disease or condition occurs by the conformal surface interaction of a polyvalent presenter with a collection of binding sites B within the subject, the conformal surface interaction resulting in the blanketing of the collection or array of target binding sites B within the subject.

5. A plurality of groups A, optionally in the form of a polyvalent presenter, used for the treatment of a disease or condition, administered to a subject such that the treatment of the disease or condition occurs by the conformal surface interaction of a polyvalent presenter with a collection of binding sites B within the subject, the conformal surface interaction resulting in the blanketing of the collection or array of target binding sites B within the subject.

6. A method for treating a disease or condition, comprising:

administering to a subject a plurality of groups A such that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a plurality of target binding sites B within the subject, said polyvalent presenter comprising a plurality of groups A attached to a framework and wherein said polyvalent presenter meets the following criteria,

i) the groups A are functional and act as a drug, alone, or in combination with the framework;

ii) the presentation of the groups A attached to the framework provide at least one, and preferably two or three, additional benefits to the interaction relative to the presentation of a single group A to a plurality of target binding sites B;

iii) the additional benefit is a synergistic benefit in that the benefit is greater than the additive benefit which would have been provided by a collection of monomers of the same group A dispersed in a homogenous solution; and

wherein said additional benefit is optionally selected from the group consisting of the provision of a sufficient biological effect at a lower concentration of Groups A, the enhancement of specificity for a targeted site versus a non-target site, positive cooperativity in binding, entropic enhancement of binding, and the production of a presenter having a low off-rate.

7. Use of a plurality of groups A for the preparation of a medicament for the treatment of a disease or condition, such that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a plurality of target binding sites B within the subject, said polyvalent presenter comprising a plurality of groups A attached to a framework and wherein said polyvalent presenter meets the following criteria,

i) the groups A are functional and act as a drug, alone, or in combination with the framework;

ii) the presentation of the groups A attached to the framework provide at least one, and preferably two or three, additional benefits to the interaction relative to the presentation of a single group A to a plurality of target binding sites B;

iii) the additional benefit is a synergistic benefit in that the benefit is greater than the additive benefit which would have been provided by a collection of monomers of the same group A dispersed in a homogenous solution; and

wherein said additional benefit is optionally selected from the group consisting of the provision of a sufficient biological effect at a lower concentration of Groups A, the enhancement of specificity for a targeted site versus a non-target site, positive cooperativity in binding, entropic enhancement of binding, and the production of a presenter having a low off-rate.

8. A plurality of groups A used for the treatment of a disease or condition in a subject, administered to a subject in such a way that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a plurality of target binding sites B within the subject, said polyvalent presenter comprising a plurality of groups A attached to a framework and wherein said polyvalent presenter meets the following criteria,

i) the groups A are functional and act as a drug, alone, or in combination with the framework;

ii) the presentation of the groups A attached to the framework provide at least one, and preferably two or three, additional benefits to the interaction relative to the presentation of a single group A to a plurality of target binding sites B;

iii) the additional benefit is a synergistic benefit in that the benefit is greater than the additive benefit which would have been provided by a collection of monomers of the same group A dispersed in a homogenous solution; and

wherein said additional benefit is optionally selected from the group consisting of the provision of a sufficient biological effect at a lower concentration of Groups A, the enhancement of specificity for a targeted site versus a non-target site, positive cooperativity in binding, entropic enhancement of binding, and the production of a presenter having a low off-rate.

9. A method for treating a disease or condition, comprising: administering to a subject at least two different types of groups (A_1 and A_2) such that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter comprising groups (A1 and A2) on a framework, wherein one of group A_1 and group A_2 is not a carbohydrate.

10. Use of at least two different types of groups (A_1 and A_2) for the preparation of a medicament for the treatment of a disease or condition, wherein said groups (A_1 and A_2) are administered to a subject such that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter comprising groups (A1 and A2) on a framework, wherein one of group A_1 and group A_2 is not a carbohydrate.

11. Two groups (A_1 and A_2) of at least two different types used for the treatment of a disease or condition, wherein said groups are administered to a subject such that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter comprising groups (A1 and A2) on a framework, wherein one of group A_1 and group A_2 is not a carbohydrate.

12. A method for treating a disease or condition, comprising:

administering to a subject at least three different types of groups (A_1, A_2, and A_3) such that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter comprising groups (A_1, A_2, and A_3) on a framework.

13. Use of at least three different types of groups (A_1, A_2, and A_3) for the preparation of a medicament for the treatment of a disease or condition, such that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter comprising groups (A_1, A_2, and A_3) on a framework.

14. Groups (A_1, A_2, and A_3), of at least three different types, used for treatment of a disease or condition, administered to a subject such that the treatment of the disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter comprising groups (A_1, A_2, and A_3) on a framework.

15. A method for facilitating the treatment of a disease or condition, comprising:

administering to a subject a plurality of groups A such that the facilitation of the treatment of a disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter having groups A on a framework;

wherein A is not a carbohydrate, a carbohydrate derivative, antibody or fragment thereof, Y is not dextran, and the polyvalent presenter is not a vaccine, or

wherein A is not a carbohydrate or polymyxin B.

16. Use of a plurality of groups A for the preparation of a medicament for the treatment of a disease or condition, such that the facilitation of the treatment of a

disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter having groups A on a framework;

wherein A is not a carbohydrate, a carbohydrate derivative, antibody or fragment thereof, Y is not dextran, and the polyvalent presenter is not a vaccine, or

wherein A is not a carbohydrate or polymyxin B.

17. A plurality of groups A used for the treatment of a disease or condition, administered to a subject such that the facilitation of the treatment of a disease or condition occurs by the interaction of a polyvalent presenter with a collection of target binding sites B, said polyvalent presenter having groups A on a framework;

wherein A is not a carbohydrate, a carbohydrate derivative, antibody or fragment thereof, Y is not dextran, and the polyvalent presenter is not a vaccine, or

wherein A is not a carbohydrate or polymyxin B.

18. The method of any one of claims 3, 6, 9 ,12, or 15, said method comprising one or more of the following optional variations:

(a) the Groups A are administered attached to a framework as part of a polyvalent presenter;

(b) the polyvalent presenters are assembled *in vivo*;

(c) the plurality of target binding sites B are present on the same interface;

(d) the plurality of target binding sites B are present on different interfaces;

(e) target binding sites B comprises more than one type of binding site;

(f) the polyvalent presenter exhibits reduced nonspecific binding of group A to binding sites non-B relative to the specific binding to binding sites B;

(g) the polyvalent presenter of groups A is effective at a lower concentration than a monovalently presented group A at the same molar concentration of A;

(h) steric inhibition, optionally non-competitive, is involved in the enhancement of the therapeutic effect;

(i) the polyvalent presenter of groups A has a higher affinity for target binding sites B than monovalently presented group A;

(j) the polyvalent presenter mediates the aggregation of the entities on which target binding sites B are present;

(k) the framework of the polyvalent presenter comprises noncovalent bonds, said framework optionally comprising a composition selected from a group consisting of a liposome, a liposcme derivative, a micelle, a colloid, a protein aggregate, and a cell;

(l) the framework of the polyvalent presenter comprises covalent bonds, said framework optionally comprising a biological particle selected from the group consisting of a sugar, a protein, a lipid, and a small molecule;

(m) the framework of the polyvalent presenter further comprises linkers, optionally an amide linker;

(n) the framework comprises a chemical moiety selected from the group consisting of a hydrolysable linkage, a dendrimer, a polymer, optionally comprising a member selected from the group consisting of a poly(ester), a poly(anhydride), a carbohydrate, a polyol, a poly(acrylate), a poly(methacrylate), a poly(ether), a poly(amino acid), poly(glutamic acid), poly(aspartic acid), dextran, dextran sulfate, poly(maleic anhydride-co-vinyl ether), poly(succinimide), poly(acrylic anhydride), poly(ethylene glycol), poly(lactic acid), poly(glycolic acid), poly(vinyl pyrrolidone), poly(styrene-maleic anhydride), poly(alpha-olefin-maleic acid), hyaluronic acid, sodium carboxymethylcellulose, chondriotin sulfate, poly(acrylamide), poly(glycerol), starch, and derivatives thereof, a copolymer, and a homopolymer, wherein said polymer is optionally attached to groups A by a covalent bond;

(o) said polyvalent presenter is water soluble, optionally in the milligram/milliliter range or in the gram/milliliter range;

(p) the volume of distribution of the polyvalent presenter in a subject is predictable;

(q) the polyvalent presenter is greater than 60 kD in molecular weight;

(r) a property of the polyvalent presenter is greater than that for monovalently presented A, said property selected from the group consisting of duration of action and therapeutic index;

(s) the polyvalent presenter has a longer half life in a specific compartment, optionally selected from the group consisting of blood, central nervous system, lungs, GI tract, and kidney, than does monovalently presented A within a subject; and

(t) the polyvalent presenter is greater than 50Å in mean hydrodynamic diameter.

19. The use of groups A as in any one of the claims 4, 7, 10, 13, and 16, said use comprising one or more of the following optional variations:

(a) the Groups A are administered attached to a framework as part of a polyvalent presenter;

(b) the polyvalent presenters are assembled *in vivo*;

(c) the plurality of target binding sites B are present on the same interface;

(d) the plurality of target binding sites B are present on different interfaces;

(e) target binding sites B comprises more than one type of binding site;

(f) the polyvalent presenter exhibits reduced nonspecific binding of group A to binding sites non-B relative to the specific binding to binding sites B;

(g) the polyvalent presenter of groups A is effective at a lower concentration than a monovalently presented group A at the same molar concentration of A;

(h) steric inhibition, optionally non-competitive, is involved in the enhancement of the therapeutic effect;

(i) the polyvalent presenter of groups A has a higher affinity for target binding sites B than monovalently presented group A;

(j) the polyvalent presenter mediates the aggregation of the entities on which target binding sites B are present;

(k) the framework of the polyvalent presenter comprises noncovalent bonds, said framework optionally comprising a composition selected from a group consisting of a liposome, a liposome derivative, a micelle, a colloid, a protein aggregate, and a cell;

(l) the framework of the polyvalent presenter comprises covalent bonds, said framework optionally comprising a biological particle selected from the group consisting of a sugar, a protein, a lipid, and a small molecule;

(m) the framework of the polyvalent presenter further comprises linkers, optionally an amide linker;

(n) the framework comprises a chemical moiety selected from the group consisting of a hydrolysable linkage, a dendrimer, a polymer, optionally comprising a member selected from the group consisting of a poly(ester), a poly(anhydride), a carbohydrate, a polyol, a poly(acrylate), a poly(methacrylate), a poly(ether), a poly(amino acid), poly(glutamic acid), poly(aspartic acid), dextran, dextran sulfate, poly(maleic anhydride-co-vinyl ether), poly(succinimide), poly(acrylic anhydride), poly(ethylene glycol), poly(lactic acid), poly(glycolic acid), poly(vinyl pyrrolidone), poly(styrene-maleic anhydride), poly(alpha-olefin-maleic acid), hyaluronic acid, sodium carboxymethylcellulose, chondriotin sulfate, poly(acrylamide), poly(glycerol), starch, and derivatives thereof, a copolymer, and a homopolymer, wherein said polymer is optionally attached to groups A by a covalent bond;

(o) said polyvalent presenter is water soluble, optionally in the milligram/milliliter range or in the gram/milliliter range;

(p) the volume of distribution of the polyvalent presenter in a subject is predictable;

(q) the polyvalent presenter is greater than 60 kD in molecular weight;

(r) a property of the polyvalent presenter is greater than that for monovalently presented A, said property selected from the group consisting of duration of action and therapeutic index;

(s) the polyvalent presenter has a longer half life in a specific compartment, optionally selected from the group consisting of blood, central nervous system, lungs, GI tract, and kidney, than does monovalently presented A within a subject; and

(t) the polyvalent presenter is greater than 50Å in mean hydrodynamic diameter.

20. The groups A of any one of the claims 5, 8, 11, 14, and 17, said treatment comprising one or more of the following optional variations:

(a) the Groups A are administered attached to a framework as part of a polyvalent presenter;

(b) the polyvalent presenters are assembled *in vivo*;

(c) the plurality of target binding sites B are present on the same interface;

(d) the plurality of target binding sites B are present on different interfaces;

(e) target binding sites B comprises more than one type of binding site;

(f) the polyvalent presenter exhibits reduced nonspecific binding of group A to binding sites non-B relative to the specific binding to binding sites B;

(g) the polyvalent presenter of groups A is effective at a lower concentration than a monovalently presented group A at the same molar concentration of A;

(h) steric inhibition, optionally non-competitive, is involved in the enhancement of the therapeutic effect;

(i) the polyvalent presenter of groups A has a higher affinity for target binding sites B than monovalently presented group A;

(j) the polyvalent presenter mediates the aggregation of the entities on which target binding sites B are present;

(k) the framework of the polyvalent presenter comprises noncovalent bonds, said framework optionally comprising a composition selected from a group consisting of a liposome, a liposome derivative, a micelle, a colloid, a protein aggregate, and a cell;

(l) the framework of the polyvalent presenter comprises covalent bonds, said framework optionally comprising a biological particle selected from the group consisting of a sugar, a protein, a lipid, and a small molecule;

(m) the framework of the polyvalent presenter further comprises linkers, optionally an amide linker;

(n) the framework comprises a chemical moiety selected from the group consisting of a hydrolysable linkage, a dendrimer, a polymer, optionally comprising a member selected from the group consisting of a poly(ester), a poly(anhydride), a carbohydrate, a polyol, a poly(acrylate), a poly(methacrylate), a poly(ether), a poly(amino acid), poly(glutamic acid), poly(aspartic acid), dextran, dextran sulfate, poly(maleic anhydride-co-vinyl ether), poly(succinimide), poly(acrylic anhydride), poly(ethylene glycol), poly(lactic acid), poly(glycolic acid), poly(vinyl pyrrolidone), poly(styrene-maleic anhydride), poly(alpha-olefin-maleic acid), hyaluronic acid, sodium carboxymethylcellulose, chondriotin sulfate, poly(acrylamide), poly(glycerol), starch, and derivatives thereof, a copolymer, and a homopolymer, wherein said polymer is optionally attached to groups A by a covalent bond;

(o) said polyvalent presenter is water soluble, optionally in the milligram/milliliter range or in the gram/milliliter range;

(p) the volume of distribution of the polyvalent presenter in a subject is predictable;

(q) the polyvalent presenter is greater than 60 kD in molecular weight;

(r) a property of the polyvalent presenter is greater than that for monovalently presented A, said property selected from the group consisting of duration of action and therapeutic index;

(s) the polyvalent presenter has a longer half life in a specific compartment, optionally selected from the group consisting of blood, central nervous system, lungs, GI tract, and kidney, than does monovalently presented A within a subject; and

(t) the polyvalent presenter is greater than 50Å in mean hydrodynamic diameter.

21. The method of any one of claims 3, 6, 9, 12, and 15,

wherein the polyvalent presenter prevents or inhibits a biological interaction selected from the group consisting of cell-cell interaction, fertilization, cell-pathogen interaction, cell-extracellular matrix interaction, pathogen-extracellular matrix interaction, neutrophil-endothelial cell interaction, inflammation, cancer metastasis, and platelet-platelet interaction, wherein said polyvalent presenter optionally modulates cellular migration or blocks a biological moiety selected from the group consisting of integrin and a platelet receptor, and

wherein said method optionally is for the treatment of a condition selected from a group consisting of acute thrombosis, a hypocoagulative state, and a hypercoagulative state.

22. The use of the groups A of any one of claims 4, 7, 10, 13, and 16,

wherein the polyvalent presenter prevents or inhibits a biological interaction selected from the group consisting of cell-cell interaction, fertilization, cell-pathogen interaction, cell-extracellular matrix interaction, pathogen-extracellular matrix interaction, neutrophil-endothelial cell interaction, inflammation, cancer metastasis, and platelet-platelet interaction, wherein said polyvalent presenter optionally modulates cellular migration or blocks a biological moiety selected from the group consisting of integrin and a platelet receptor, and

wherein said use optionally is for the preparation of a medicament for the treatment of a condition selected from the group consisting of acute thrombosis, a hypocoagulative state, and a hypercoagulative state.

23. The groups A of any one of claims 5, 8, 11, 14, and 17,

wherein the polyvalent presenter prevents or inhibits a biological interaction selected from the group consisting of cell-cell interaction, fertilization, cell-pathogen interaction, cell-extracellular matrix interaction, pathogen-extracellular matrix interaction, neutrophil-endothelial cell interaction, inflammation, cancer metastasis, and platelet-platelet interaction, wherein said polyvalent presenter optionally modulates cellular migration or blocks a biological moiety selected from the group consisting of integrin and a platelet receptor, and

wherein said groups optionally are used for the treatment of a condition selected from the group consisting of acute thrombosis, a hypocoagulative state, and a hypercoagulative state.

24. A method for inhibiting conception, comprising:

administering to a subject a plurality of groups A, wherein A optionally comprises GlcNAc, such that inhibition of conception occurs by the interaction of a polyvalent presenter with a plurality of target binding sites B, said polyvalent presenter having Groups A on a framework.

25. Use of a plurality of groups A for the preparation of a medicament for inhibiting conception, comprising:

administering to a subject a plurality of groups A, wherein A optionally comprises GlcNAc, such that inhibition of conception occurs by the interaction of a polyvalent presenter with a plurality of target binding sites B, said polyvalent presenter having Groups A on a framework .

26. A plurality of groups A used for inhibiting conception, administered to a subject, wherein A optionally comprises GlcNAc, such that inhibition of conception occurs

by the interaction of a polyvalent presenter with a plurality of target binding sites B, said polyvalent presenter having Groups A on a framework.

27. The method of any one of claims 3, 6, 9, 12, and 15 wherein the polyvalent presenter prevents a biological interaction selected from the group consisting of cell-pathogen attachment and pathogen-extracellular matrix attachment, wherein the pathogen is optionally selected from a group consisting of a bacteria, a fungus, a virus, and a parasite.

28. The use of groups A of any one of claims 4, 7, 10, 13, and 16 wherein the polyvalent presenter prevents a biological interaction selected from the group consisting of cell-pathogen attachment and pathogen-extracellular matrix attachment, wherein the pathogen is optionally selected from a group consisting of a bacteria, a fungus, a virus, and a parasite.

29. The groups A of any one of claims 5, 8, 11, 14, and 17 wherein the polyvalent presenter prevents a biological interaction selected from the group consisting of cell-pathogen attachment and pathogen-extracellular matrix attachment, wherein the pathogen is optionally selected from a group consisting of a bacteria, a fungus, a virus, and a parasite.

30. A pharmaceutical composition for polyvalently presenting an agent for therapy, comprising:

a polyvalent presenter having a formula as follows:

$$(Y)\text{-}(X\text{-}A)_n$$

wherein Y is a framework, optionally polymeric, X is a direct bond or a linker group, said linker group optionally being an independent moiety and not a part of Y or A, A is a presented functional group, and

n is an integer greater than ten,

optionally further comprising one or more of the following variations:

(a) said integer is selected and the (X-A) moieties are attached to Y such that the polyvalent presenter conforms to a collection of target binding sites B upon administration to a subject, optionally blanketing an array of target binding sites B upon administration to a subject;

(b) said integer is selected such that the polyvalent presenter conforms to a collection of target binding sites B upon administration to a subject; and

(c) said composition optionally further comprises a pharmaceutically acceptable carrier.

31. A conforming polyvalent presenter, comprising:

a plurality of groups A on a framework forming a polyvalent presenter, said polyvalent presenter being conformed such that it blankets a collection of target binding sites within a subject.

32. A pharmaceutical composition, comprising:

a collection of at least two different polyvalent presenters, and

a pharmaceutically acceptable carrier.

33. A method of modulating adhesion between a plurality of a surface-bound biological molecule and a plurality of a second molecule, said method comprising:

providing in combination a plurality of a surface-bound biological molecule and a polyvalent presenter, optionally a non-saccharide and having an enhancement factor β greater than about 10^2, comprising a framework to which is attached a plurality of a third molecule wherein said third molecule modulates the adhesion between said surface-bound biological molecule and said second molecule by simultaneous binding of said third molecule to a portion of the plurality of said surface-bound biological molecule and by sterically blocking by said framework of a portion of the plurality of said surface bound biological molecule to which said third molecule is not bound, wherein said adhesion optionally occurs between a cell and an entity selected from the group consisting of a bacterium, a virus, an influenza virus, a second cell, and a polyvalent molecule.

34. A polyvalent presenter selected from the group consisting of pAA(Gal-β), pAA(Gal-α), pBMA(Gal-β), pBMA(Gal-α), and pAA(GlcNAc-β).

35. A method for preventing the adhesion of ricins to erythrocytes, comprising contacting said ricins with an effective amount of a polyvalent presenter selected from the group consisting of pAA(Gal-β), pAA(Gal-α), pBMA(Gal-β) and pBMA(Gal-α).

36. A method for preparing a polyvalent presenter selected from the group consisting of pAA(Gal-β) and pBMA(Gal-β), said method comprising:

(a) reacting Gal-β_O-L_1NH_2 with poly(*N*-acryloyloxysuccinimide) or poly(butadiene-co-maleic anhydride) and

(b) quenching said reaction.

37. A method for preparing a polyvalent presenter selected from the group consisting of pAA(Gal-α) and pBMA(Gal-α), said method comprising:

(a) reacting Gal-α_C-L_2NH_2 with poly(*N*-acryloyloxysuccinimide) or poly(butadiene-co-maleic anhydride) and

(b) quenching said reaction.

38. A method for inhibiting fertilization in a subject comprising administering to said subject an effective amount of a polyvalent presenter that is pAA(GlcNAc-β).

39. Use of a polyvalent presenter that is pAA(GlcNAc-β) for the preparation of a composition for the inhibition of fertilization.

40. A polyvalent presenter A used for inhibiting fertilization, wherein said polyvlalent presenter is pAA(GlcNAc-β) and is administered in an amount effective for inhibiting fertilization in a subject.

41. A method for preparing a polyvalent presenter that is pAA(GlcNAc-β), said method comprising:

(a) reacting GlcNAc-β-L_1NH_2 with poly(*N*-acryloyloxysuccinimide) and

(b) quenching said reaction.

42. A method of making an array of polyvalent presenters, said method comprising:

(a) delivering a first activated framework component of a first polyvalent presenter and a first activated framework component of a second polyvalent presenter to first and second reaction vessels;

(b) delivering a first functional group component of said first polyvalent presenter and a first functional group component of said second polyvalent presenter to said first and second reaction vessels;

(c) allowing the activated components to react thereby forming an array of at least two different polyvalent presenters;

said method optionally further comprising one or more of the following steps:

(d) delivering a first spacer group of said first polyvalent presenter to said first reaction vessel;

(e) delivering a first spacer group of said second polyvalent presenter to said reaction vessel;

(f) delivering an ancillary group of said first polyvalent presenter to said first reaction vessel;

(g) delivering an ancillary group of said second polyvalent presenter to said second reaction vessel;

said method comprising one or more of the following optional variations:

(1) repeating the steps, wherein "n" number of functional groups of said first and said second polyvalent presenters are delivered to said respective first and second reaction vessels;

(2) repeating the steps, wherein "n" number of spacer groups of said first and said second polyvalent presenters are delivered to said respective first and second reaction vessels;

(3) repeating the steps, wherein "n" number of ancillary groups of said first and said second polyvalent presenters are delivered to said respective first and second reaction vessels.

43. A polyvalent presenter or array of polyvalent presenters preparable by any one of the methods of claim 1, 2, 36, 37, 41, or 42.

FIGURE 1

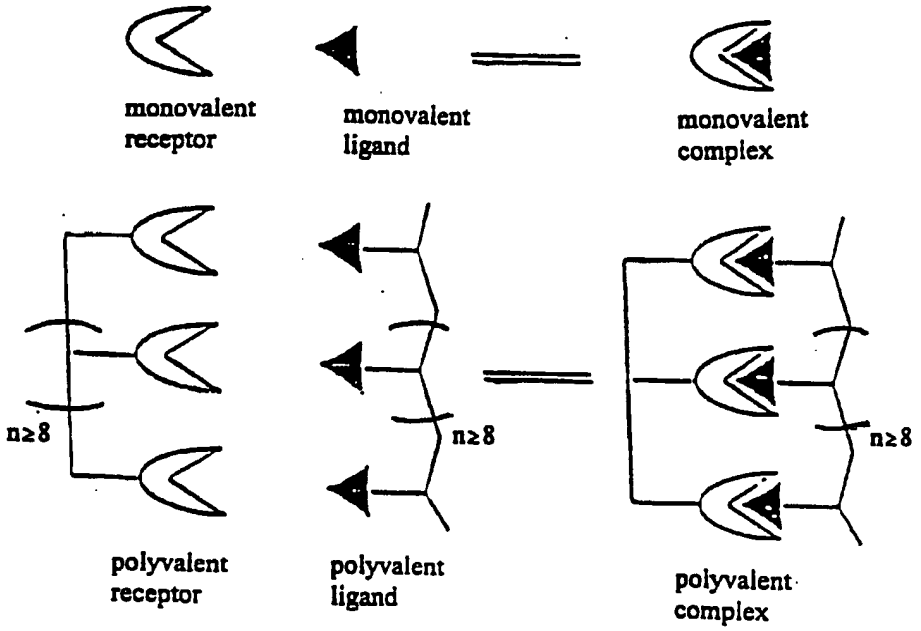

monovalent receptor
monovalent ligand
monovalent complex
(1)
n≥8
n≥8
n≥8
polyvalent receptor
polyvalent ligand
polyvalent complex
(2)

FIGURE 2

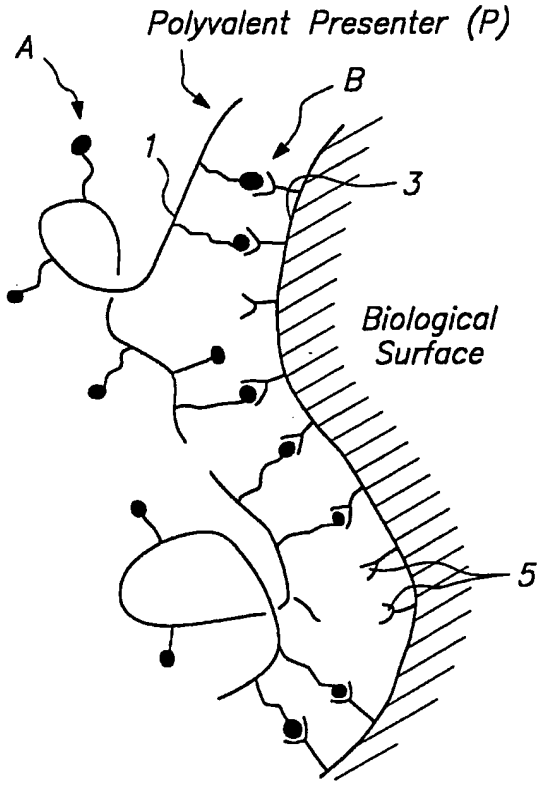


FIGURE 3

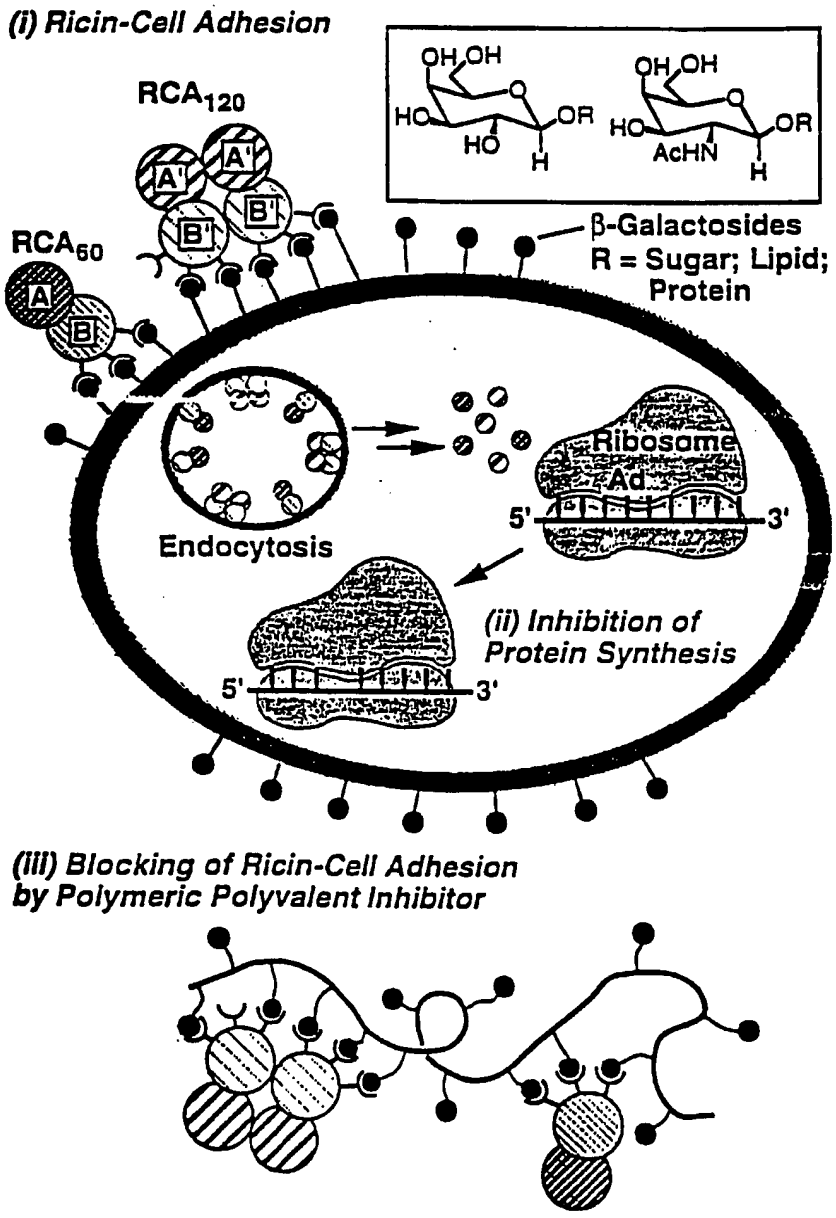

(i) Ricin-Cell Adhesion
RCA120
RCA60
A'
B'
A
B
β-Galactosides
R = Sugar; Lipid; Protein
Endocytosis
Ribosome
Ad
5'
3'
(ii) Inhibition of Protein Synthesis
(iii) Blocking of Ricin-Cell Adhesion by Polymeric Polyvalent Inhibitor

FIGURE 4

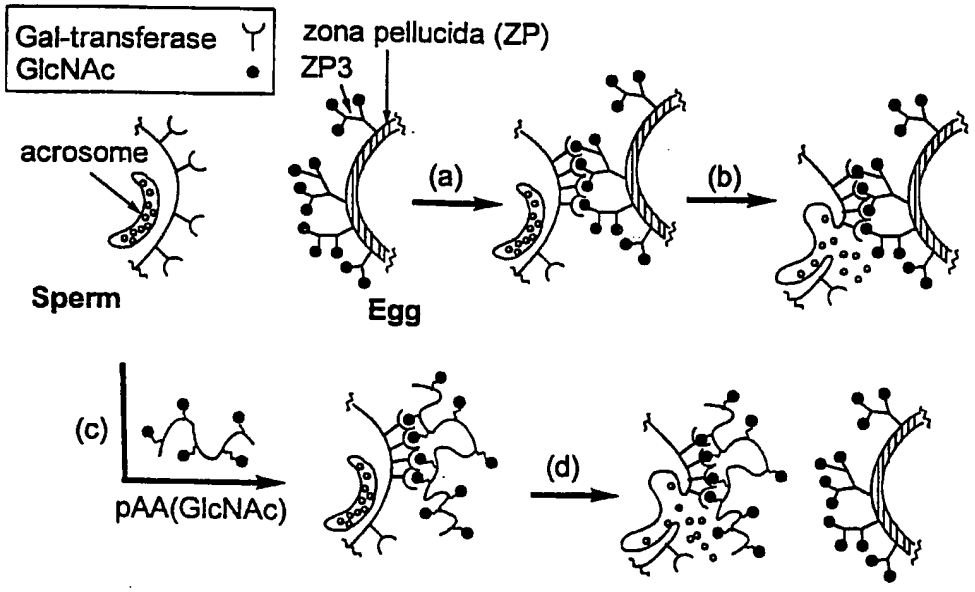
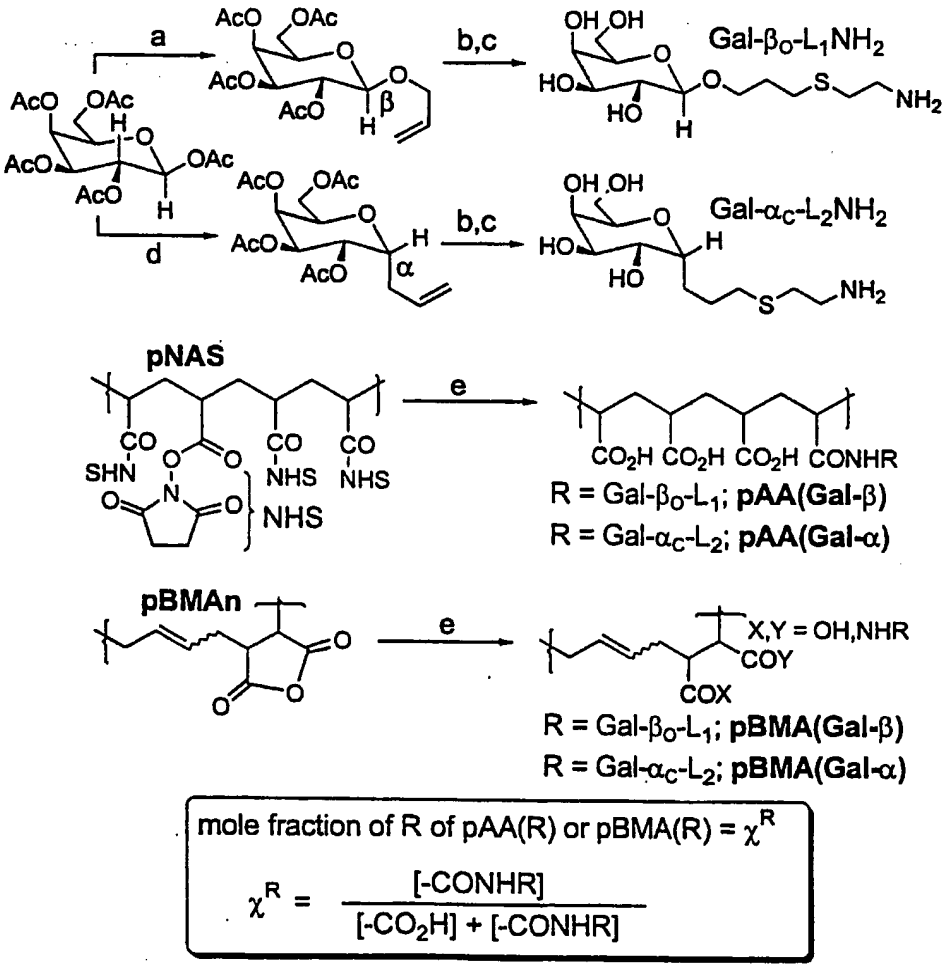

Gal-transferase
GlcNAc
zona pellucida (ZP)
ZP3
acrosome
Sperm
Egg
(a)
(b)
(c)
pAA(GlcNAc)
(d)

FIGURE 5



mole fraction of R of pAA(R) or pBMA(R) = χ^R

$$\chi^R = \frac{[\text{-CONHR}]}{[\text{-CO}_2\text{H}] + [\text{-CONHR}]}$$

FIGURE 6

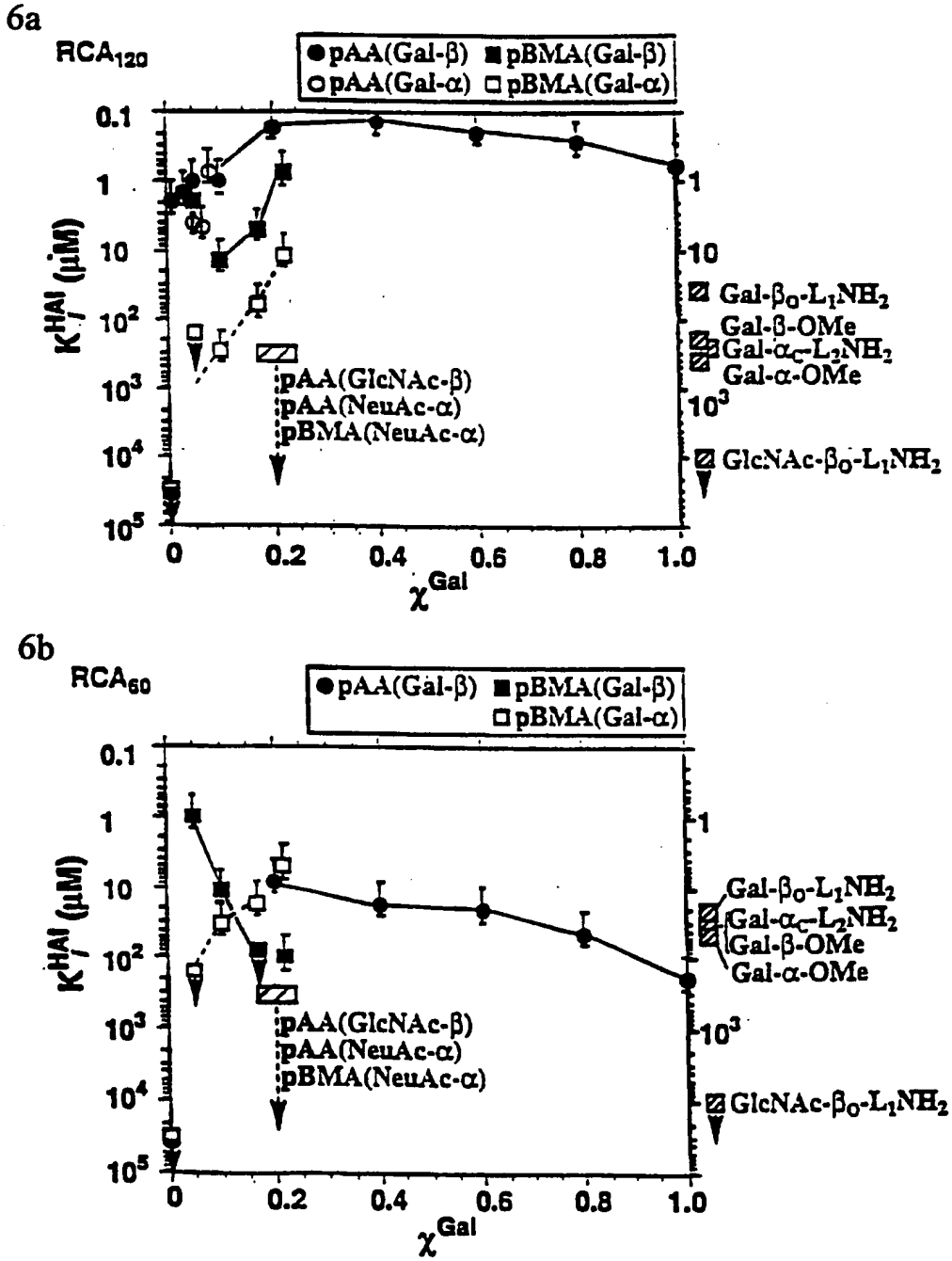
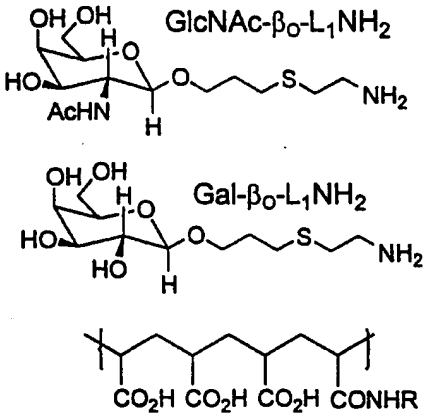

6a
RCA120
pAA(Gal-β) pBMA(Gal-β)
pAA(Gal-α) pBMA(Gal-α)
K_I^{HAI} (μM)
0.1
1
10
10^2
10^3
10^4
10^5
pAA(GlcNAc-β)
pAA(NeuAc-α)
pBMA(NeuAc-α)
Gal-β-OMe
Gal-α-OMe
10^3
0
0.2
0.4
0.6
0.8
1.0
χ^{Gal}
6b
RCA60
pAA(Gal-β) pBMA(Gal-β)
pBMA(Gal-α)
K_I^{HAI} (μM)
0.1
1
10
10^2
10^3
10^4
10^5
pAA(GlcNAc-β)
pAA(NeuAc-α)
pBMA(NeuAc-α)
Gal-β-OMe
Gal-α-OMe
10^3
0
0.2
0.4
0.6
0.8
1.0
χ^{Gal}

FIGURE 7



R = GlcNAc-β$_O$-L$_1$; **pAA(GlcNAc; χ)**
(χ^{GlcNAc} = 0.2, 0.3, 0.6, 0.8, and 1.0)
R = Gal-β$_O$-L$_1$; **pAA(Gal; χ)**
(χ^{Gal} = 0.3, 0.6, and 1.0)

χ^R = mole fraction of R of pAA(R)

$$\chi^R = \frac{[\text{-CONHR}]}{[\text{-CO}_2\text{H}] + [\text{-CONHR}]}$$

FIGURE 8

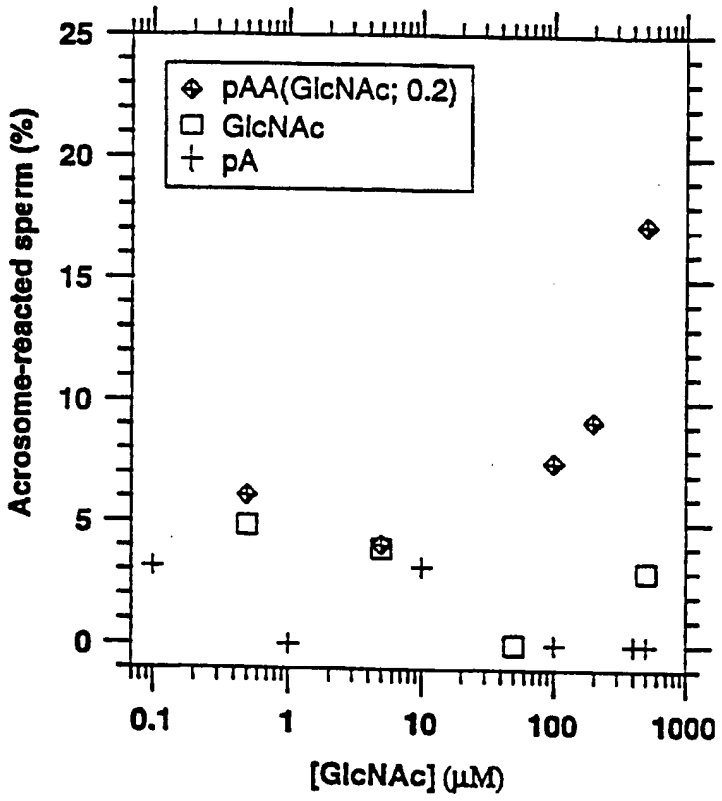

pAA(GlcNAc; 0.2)
GlcNAc
pA
Acrosome-reacted sperm (%)
25
20
15
10
5
0
0.1
1
10
100
1000
[GlcNAc] (μM)

FIGURE 9

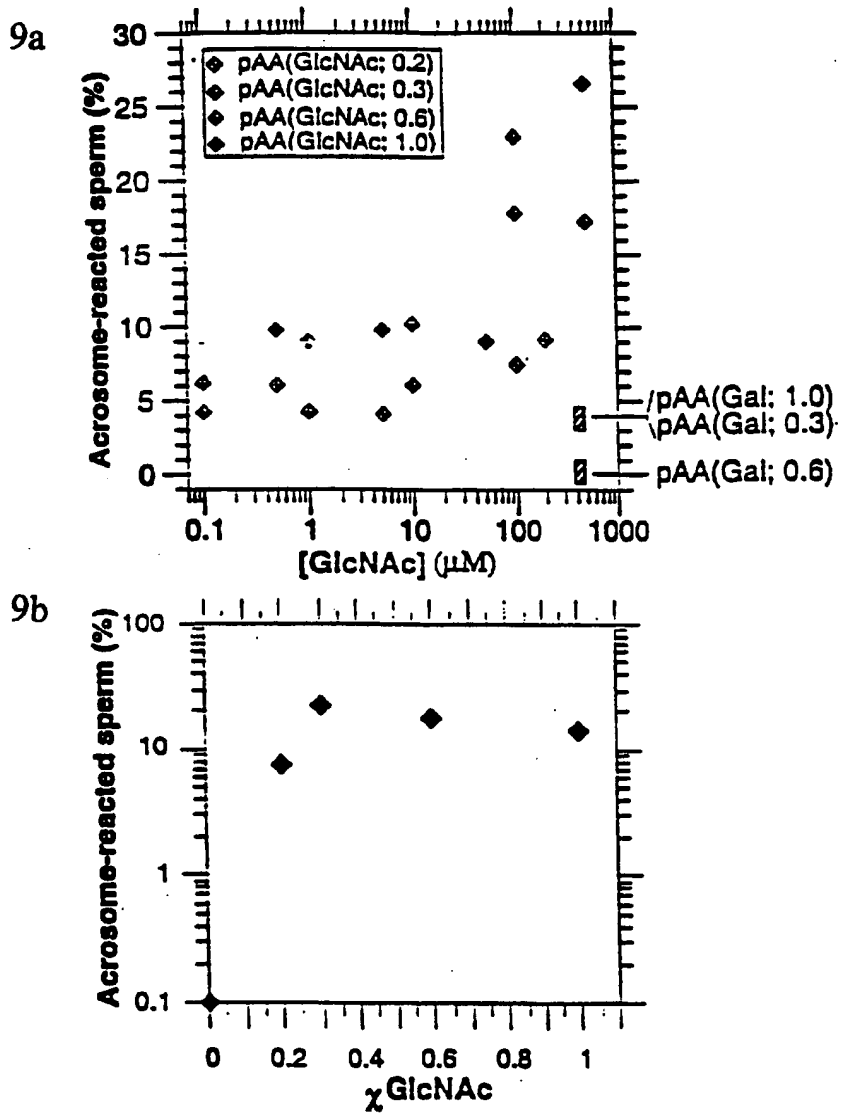

9a
Acrosome-reacted sperm (%)
30
25
20
15
10
5
0
pAA(GlcNAc; 0.2)
pAA(GlcNAc; 0.3)
pAA(GlcNAc; 0.6)
pAA(GlcNAc; 1.0)
/pAA(Gal; 1.0)
\pAA(Gal; 0.3)
pAA(Gal; 0.6)
0.1
1
10
100
1000
[GlcNAc] (μM)
9b
Acrosome-reacted sperm (%)
100
10
1
0.1
0
0.2
0.4
0.6
0.8
1
χGlcNAc

FIGURE 10

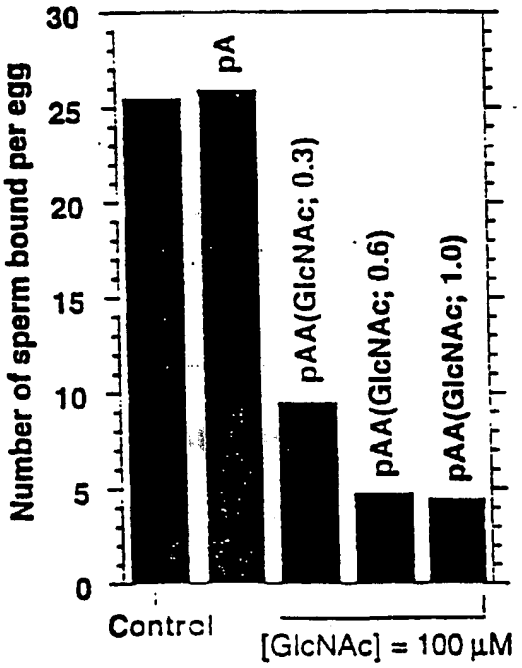
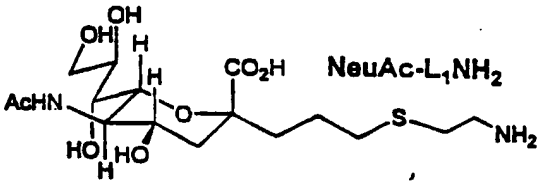
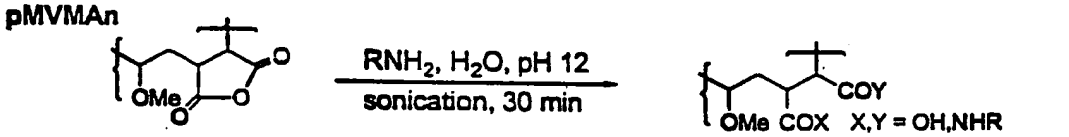

Number of sperm bound per egg
30
25
20
15
10
5
0
pA
pAA(GlcNAc; 0.3)
pAA(GlcNAc; 0.6)
pAA(GlcNAc; 1.0)
Control
[GlcNAc] = 100 µM

FIGURE 11

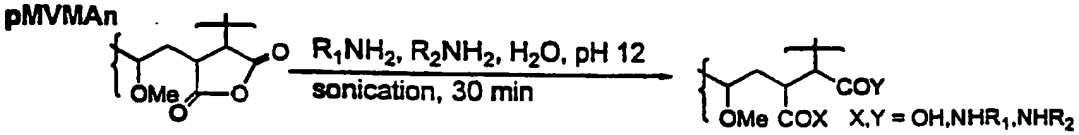


11a



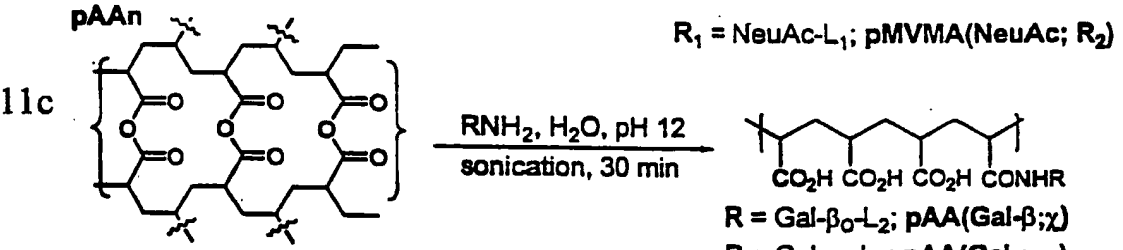
R = NeuAc-L_1; **pMVMA(NeuAc;χ)**

11b



R_1 = NeuAc-L_1; **pMVMA(NeuAc; R_2)**

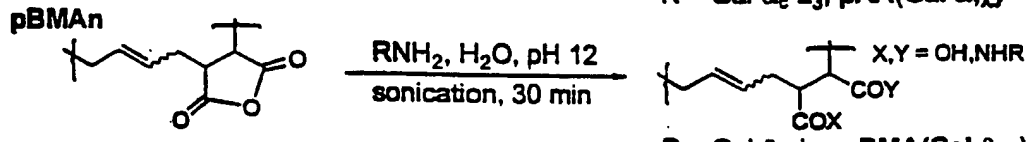
11c



R = Gal-β_O-L_2; **pAA(Gal-β;χ)**

R = Gal-α_c-L_3; **pAA(Gal-α;χ)**

11d



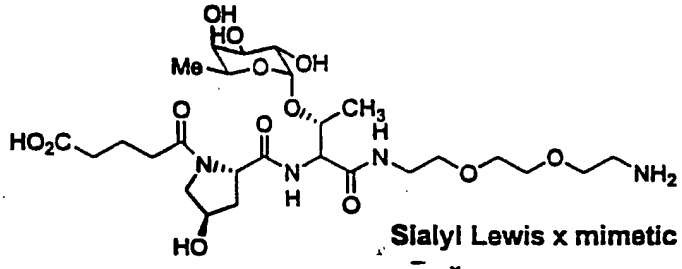
R = Gal-β_O-L_2; **pBMA(Gal-β;χ)**

R = Gal-α_C-L_3; **pBMA(Gal-α;χ)**

χ = mole fraction of R of pAA(R;χ) or pBMA(R;χ)

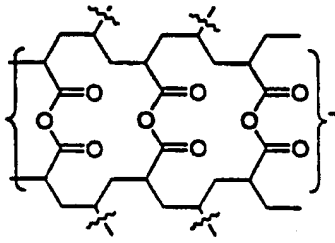
$$\chi^R = \frac{[\text{-CONHR}]}{[\text{-CO}_2\text{H}] + [\text{-CONHR}]}$$

FIGURE 12



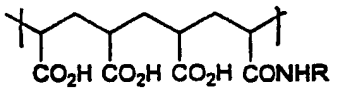
Sialyl Lewis x mimetic
($\bar{S}Le^x$-LNH_2)

12a



pAAn

SLe^x-NH_2, H_2O, pH 12
sonication, 30 min

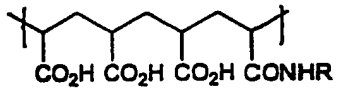


R = SLe^x-LNH_2; **pAA(SLe^x;χ)**

12b

pAAn

Bacitracin, H_2O, pH 12
sonication, 30 min



R = Bacitracin; **pAA(bacitracin;χ)**

χ = mole fraction of R of pAA(R;χ), pMVMA(R;x), or pBMA(R;χ)

$$\chi^R = \frac{[\text{-CONHR}]}{[\text{-CO}_2\text{H}] + [\text{-CONHR}]}$$